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(54) Title: SACCHAROPEPTIDES AND DERIVATIVES THEREOF

(57) Abstract

The present invention describes glycomimetic saccharopeptides of formula (I): W-$(X)_n$-Y-[$(X)_n$-W-$(X)_n$-Y]$_m$-$(X)_n$-W, wherein W is independently selected from the group consisting of a) saccharides; b) aryl, aralkyl; c) alkyl of 1 to 8 carbon atoms, optionally substituted; d) cyclic alkyl or 5-7 carbon atoms, heterocyclic alkyl. Y is independently selected from the group consisting of -NR^3-C(O)- and -C(O)-NR^3-; X is a difunctional or polyfunctional group selected from the group consisting of a) aryl, aralkyl; b) alkyl of 1-8 carbon atoms, optionally substituted with 1-3 substituents. R is -H, or lower alkyl, lower aryl, and lower aralkyl; R' is independently selected from the group consisting of -H, lower alkyl of 1-4 carbon atoms, aralkyl of 2 to 19 carbon atoms, and -C(O)R"; R" is lower alkyl of 1 to 4 carbon atoms; and R^3 is selected from the group consisting of -H, alkyl of 1-8 carbon atoms, and aralkyl of 5-8 carbon atoms; and pharmaceutically acceptable salts thereof.

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DESCRIPTION

SACCHAROPEPTIDES AND DERIVATIVES THEREOF

This application is a continuation-in-part of Serial Number 08/438,669, filed May 10, 1995, which is incorporated by reference in its entirety.

Field of the Invention

The present invention relates to a novel class of carbohydrate derivatives, saccharopeptides, containing amide bonds, and to methods of preparing these saccharopeptides, methods of using these saccharopeptides to treat certain diseases and to purify proteins.

Background of the Invention

Naturally occurring carbohydrates occur either in the form of free sugars, or as monosaccharide units linked to other components, such as other sugars (forming oligo- and polysaccharides), proteins (glycoproteins), lipids (glycolipids) or other organic molecules (*e.g.* nucleosides, steroid glycosides, flavanoids *etc.*). The sugars (mono-saccharides) are attached to each other or to other types of compounds by glycosidic linkage. Most commonly this is an O-glycosidic linkage, but heteroatom substitutions (S,N,C) both exocyclically and endocyclically are also encountered.

Carbohydrates possess a number of pharmacologic activities. Theses activities include binding to selectins which results in neutrophil recruitment to

sites of inflammation. Another pharmacologic activity is the binding of the carbohydrates, heparin and heparin sulfate, to proteins on cell surfaces to modulate a variety of cellular functions including morphology, growth, and migration. Heparin and heparin derivatives also possess useful antithrombotic activity.

Additionally, heparin and heparan sulfate interact with growth factors and heparinases to mediate angiogenesis and tumor growth. Carbohydrates are also important cell surface ligands by which viruses attach to and infect cells. In addition to other activities, carbohydrates are also important moieties of antibiotics and immune modulators. These pharmocalogic activities are described in more detail below.

Glycomimetic Inhibitors of Carbohydrate-Mediated Leukocyte Adhesion

A large body of data has been accumulated that establishes a family of receptors, the selectins (LECAMs) in certain diseases including cancer, autoimmunity, and in the inflammatory response. The three known members of this family, L-Selectin (LECAM-1, LAM-1, gp90MEL), E-Selectin (LECAM-2, ELAM-1) and P-Selectin (LECAM-3, GMP-140, PADGEM), each contain a domain with homology to the calcium-dependent Lectins (C-lectins), an EGF-like domain, and several complement binding protein-like domains (Bevilacqua et al., Science (1989) 243:1160-1165; Johnston et al., Cell (1989) 56:1033-1044; Lasky et al., Cell (1989) 56:1045-1055; Tedder et al ., J. Exp. Med. (1989) 170:123-133, Dasgupta et al . , Exp. Opin. Invest Drugs (1994) 3(7):709). It has been proposed that the

selectins bind to particular carbohydrate ligands and that this accounts for their biological activity. Thus, drugs that interfere with or prevent binding of the ligands to the selectins are useful medicaments for treating a variety of diseases.

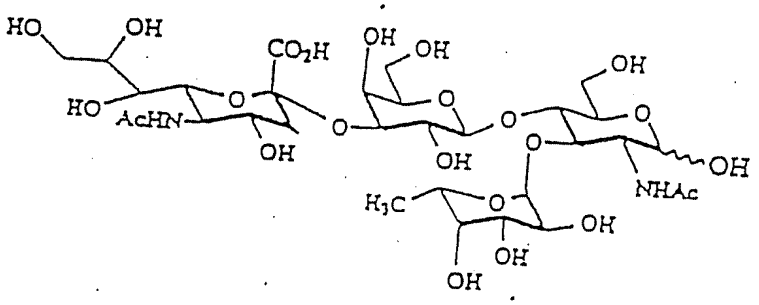
The selectin family of adhesion molecules participate in acute inflammation by initiating leukocyte rolling on activated endothelial cells. This is particularly evident in studies of ischemia reperfusion injury, where P-selectin appears to be important in neutrophil recruitment to damaged tissue. Recently, Buerke et al. have demonstrated the important role of selectins in inflammatory states such as ischemia-reperfusion injury in cats (Buerke, M. et al., J. Clin. Invest. (1994) 93:1140). Turunen et al. have demonstrated the role of sLeX and L-selectin in site-specific lymphocyte extravasation in renal transplants during acute rejection (Turunen, J.P. *et al.*, Eur. J. Immunol. (1994) 24:1130). P-selectin has been shown to be centrally involved particularly as related to acute lung injury. Mulligan et al. have reported strong protective effects using anti-P-selectin antibody in a rodent lung injury model. (Mulligan, M. S. et al., J. Clin. Invest., (1991) 90:1600, Mulligan, M. S. et al., Nature (1993) 364:149). A central role of P-selectin in inflammation and thrombosis has been demonstrated by Palabrica et al. (Palabrica, T. et al., Nature (1992) 359:843).

E-selectin is implicated in initial neutrophil extravasation in response to infection and injury. (Bevilacqua, *et al.*, Science (1989) 243:1160). Indeed, Gundel et al. have shown that antibody to E-selectin

blocks the influx of neutrophils in a primate model of asthma and thus is beneficial for preventing airway obstruction resulting from the inflammatory response. (Gundel R. H. et al. , J. Clin. Invest. (1991) 88:1407).

The presence of L-selectin and E- or P-selectin ligands on mononuclear cells has implicated these receptor-ligand interactions in chronic inflammation. This has been supported by the finding of chronic expression of E-selectin in dermatologic conditions, and P-selectin expression on joint synovial endothelium derived from rheumatoid arthritis patients. L. Lasky Annu. Rev. Biochem. 64:113-39 (1995); "Selectin Family of Adhesion Molecules" by Michael Forrest and James C. Paulson in Physiology and Pathophysiology of Leukocyte Adhesion, Ed. by D. Niel Grangier and Deert SchmidSchonbein, Oxford University Press, N.Y., N.Y. (1995).

It is known that selectins bind to certain carbohydrates. E-selectin has a Lectin like domain that recognizes the Sialyl Lewis x (sLeX) tetrasaccharide epitope as shown below in Structure III.

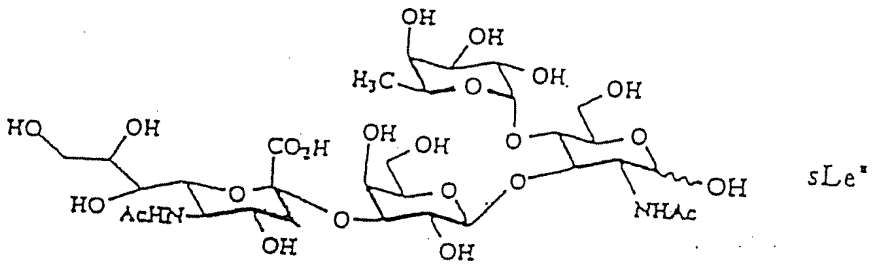


sLe^x

III

The ability of sLe^x to bind E-selectin is described by Lowe et al., Cell (1990) 63:475; Phillips et al., Science (1990) 250-1130; Walz et al., Science (1990) 250:1132; and Tyrrell et al ., Proc. Natl. Acad. Sci. USA (1991) 88:10372.

It has also been shown (Berg et al., J. Biol. Chem. (1991) 265:14869; Handa et al ., Biochem. Biophys. Res. Commun. (1991) 181:1223) that both E-selectin and P-selectin recognize the isomeric tetrasaccharide sLe^a shown below as Structure IV.

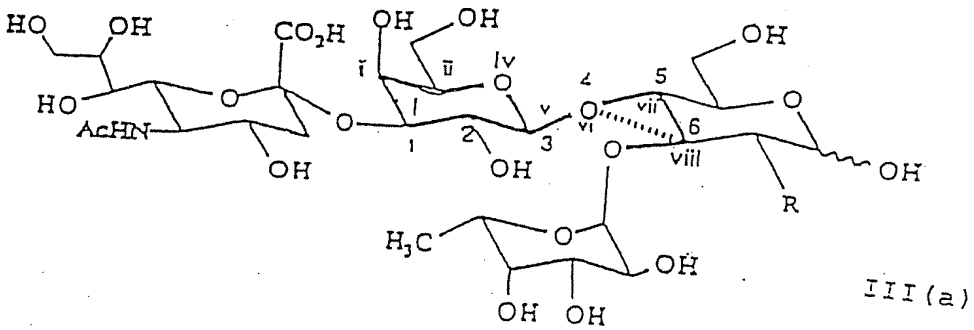


IV

L and P-selectin also bind to sLe^x containing ligands, although these selectins have specificity toward a wider variety of natural ligands containing sialylated and sulfated Le^x, and Le^a structures as well as other sulfated or charged carbohydrates (Varki et al. Proc. Nat'l Acad. Sci. USA 91:7390-7397 (1994); and Rosen, *et al.* Current Opinion in Cell Biology (1994) 6:663-673). sLe^x and sLe^a share a structural similarity in their three dimensional arraragements. (Berg, E.L., *et al.*, J. Biol. Chem. (1991) 266:14865-14872). Specifically, it was observed that sialic acid and fucose, two functional

epitopes in these tetrasaccharides, are juxtaposed in space in a way suitable for recognition by the selectins. Most importantly, for both tetrasaccharides 4 to 12 atoms associated with the lactose core of the tetrasaccharides were identified that functionally separate sialic acid from fucose. It was postulated that replacement of these atoms would lead to glycomimetic compounds, such as those described and claimed herein, that maintain their selectin binding activity. While 4 to 12 is the preferred number of atoms, most preferred is 6 to 8 atoms as shown in the figure below. The number of atoms refers to the number of atoms between the O-glycoside of sialic acid and the O-glycoside of fucose.

For instance, a close structural examination of sLe^x (shown in III) or a modification thereof wherein R = OH (sLe^xGlc) indicates that the epitopes i.e., α -Neu5Ac and L-Fucose, are linked through six atoms (Nos. 1-6) or eight atoms (Nos. i-viii) as shown in Structure III (a) below wherein R is NHAc or OH.



III(a)

Based on this discovery, it was deduced that the corresponding epitopes on the Lectin domain of the selectins, are spaced in a similar three-dimensional configuration such that maintenance of the 6 to 8 atoms in the ligand structure would yield active ligands that are

markedly different in structure from the naturally occurring ligand.

It was also shown that sLe^x and sLe^a present the fucose and sialic acid functionalities in a special relationship placing them on a single face with a spacing of 10-12 Å measured between the carbonyl carbon of the carboxylic acid on sialic acid and the C-3 of fucose. (Rao *et al.* J. Biol. Chem. (1994) 269(31):19663). Certain compounds of the present invention possess an acid functionality mimic which is preferably 8-14 Å, and more preferably 9-11 Å from a fucose or fucose mimic. This distance is measured from the carbonyl carbon of the acid mimic to the C-3 carbon of fucose or its equivalent on its mimic. These two functionalities are linked through a peptide bond.

The glycomimetic compounds of the present invention offer considerable diversity and facility in terms of attachment of suitable groups to satisfy the spacial requirements for selectin ligand binding.

Considering the obvious medical importance of selectin ligands, significant effort has been, and continues to be expended to identify the critical physical/chemical parameters associated with selectin ligands that enhance, or that are required for their activity (DeFrees, S.A., et al., J. Am. Chem. Soc., (1993) 115:7549). In no small part this effort is being driven by the need to have selectin ligands that are inexpensive to produce (see U.S. Patent 5,296,594 issued March 22, 1994; Allanson, N.M. et al., Tetrahedron Lett., (1993) 34:3945; Musser, J.H. et al., Current Pharmaceutical Design (1995) 221-232). It is generally thought that it will be

prohibitively expensive to commercially produce naturally occurring sLe^x by either enzymatic or chemical synthesis because of the number of sophisticated reactions involved. A number of papers have published on sLe^x mimetics. U.S. Patent 5,508,387 issued April 16, 1996; B.N. Rao et al, Med. Chem. Res(1991) 1:1-8; B.N. Rao et al, J. Biol. Chem. (1994) 269(31): 19663-19666; T. Uchiyama et al, J. Am. Chem. Soc. (1995) 117: 5395-5396; J.Y. Ramphal et al J. Med. Chem. (1994) 37: 3459-3463; D.E. Levy et al, Ann. Reports Med. Chem (1994) 29: 215-224; B. Dupre *et al.*, Bioorg. Med. Chem. Let (1996) 6(5): 569-572; A.A. Birkbeck, et al, Bioorg. Med. Chem. Let (1996) 5(22): 2637-2642; U. Sprengard et al, Bioorg. Med. Chem. Let (1996) 6(5): 509-514; S.-H. Wu et al, Ang. Chem. Int Ed. Engl (1996) 35(1): 96-98; and Ragan et al, Bioorg. Med. Chem. Let (1994) 4(21): 2563-2566. Therefore, approaches such as those described in this invention, toward non-oligosaccharide glycomimetics of sLe^x have the potential to provide more potent, cost effective, stable and bioavailable drug candidates.

Heparin and Heparan Sulfate Sequence Mimic Therapeutics

Heparin and heparan sulphate (HS) constitute a class of glycosaminoglycans (GAGs) which can modulate a plethora of cellular functions (e.g., growth, morphology, migration, etc.) by interacting with extracellular matrix (ECM) proteins (Kjéllen and Lindahl, (1991) Annu. Rev. Biochem. 60:443), growth factors (Bobik and Campbell, Pharmacological Rev. (1993) 45:1, growth factor high-affinity receptors (Spivak-Kroizman *et al.*, Cell (1994) 79:1015), enzymes (including leukocyte proteases) (Redini

et al., Biochem. J. (1988) 252:515) and proinflammatory mediators (Miller and Krangel, Critical Rev. Immunol. (1992) 12:17). These complex carbohydrates are most often found attached to serine residues of proteins to form proteoglycans. Heparan sulphate proteoglycans (HSPGs) are distributed in basement membranes and stromal matrices, and they are associated with almost all cell surfaces. They are also found in amyloid plaques from Alzheimer's patients. Snow, *et al.* Neurobiology Aging (1989) 10:481-497. Through their HS chains, they interact with numerous factors to regulate cell and tissue-specific events such as blood coagulation (Marcum and Rosenberg, (1989) "The Biochemistry, Cell Biology, and Pathophysiology of Anticoagulantly Active Heparin-like Molecules of the Vessel Wall" in Heparin, Lane and Lindahl (eds), CRC Press, Inc., Boca Raton, FL pp. 275-294), leukocyte activation (Tanaka *et al.*, Immunol. Today (1993) 14:111), cell motility (Makabe *et al.*, J. Biol. Chem. (1990) 265:14270) and cell proliferation (Rapraeger *et al.*, Science (1991) 252:1705). Since many of these properties are not related to the anticoagulant activities, there is considerable interest in developing therapeutics based on the non-anticoagulant activities of heparin (U. Lindahl et al., Thrombosis Res. (1994) 75(1); 1-32, D.A. Lane and L. Adams, N. Engl. J. Med. (1993) 329(2); 129-130, D.J. Tyrrell et al, TiPS (1995) (16); 198-204.). The biological properties of HS and heparin-related structures make them attractive leads for the development of novel therapeutics.

Heparin is a more biosynthetically mature form of the general heparan sulfate class of glycosaminoglycans.

The possibility that small defined sequences of heparan sulfate or heparin possess specific biological properties is supported by the well characterized pentasaccharide sequence responsible for the heparin-antithrombin III (ATIII) interaction, and the minimal hexasaccharide sequence responsible for the heparin-bFGF interaction. These results suggest that carbohydrate-derived and heparin sequence mimics could be viable strategies to produce antithrombotic agents for treating thrombotic disorders, and bFGF antagonists for inhibiting angiogenesis and cancer tumor growth.

Heparin is a widely used anticoagulant and antithrombotic therapeutic. Heparin functions as an anticoagulant by catalytically increasing by approximately 1000 fold the rate at which certain circulatory serine protease inhibitors (serpins) bind to and inhibit serine proteases involved in coagulation (M.-C. Bourin and U. Lindahl, Biochem. J. (1993) 289; 313-330). Structural sequences in heparin mediate the formation of a complex with these serpin cofactors that is responsible for the catalytic effect. In this manner, the serpin antithrombin III (AT III) more effectively inhibits the proteases factor IIa and factor Xa while the serpin heparin-cofactor II (HC II) more effectively inhibits the protease factor IIa (thrombin). Heparin binds to AT III by a specific well defined pentasaccharide sequence contained in the heparin polysaccharide chain, while binding of heparin to HC II is via a relatively nonspecific, delocalized electrostatic interaction.

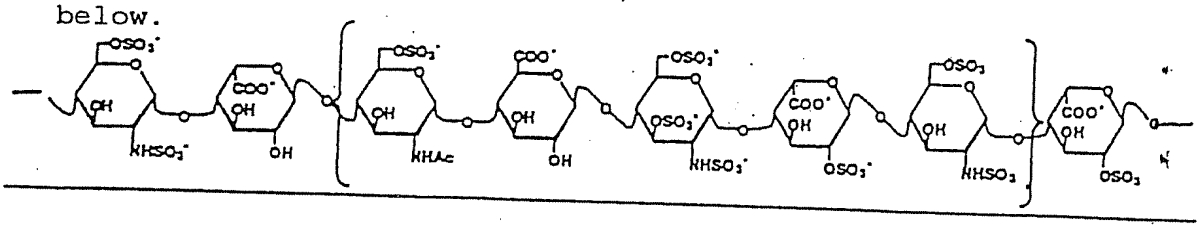
Low molecular weight heparins, hereinafter "LMW heparin", (Low Molecular Weight Heparins in Clinical

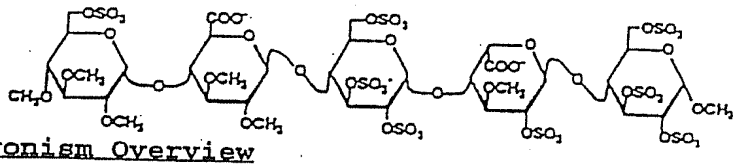
Practice, C. Doutremepuich, Ed., Marcel Dekker, Inc., 1992) and synthetic heparin sequences (van Boeckel and Petitou, Ang. Chem. (1993), 32(12):1671-1818) have been investigated as an approach to avoid certain complications associated with the clinical use of heparin such as bleeding and heparin-induced thrombocytopenia, and in more chronic use, osteoporosis.

The LMW heparins have proven to be clinically efficacious for antithrombotic indications (D. Green et al, Pharmacological Reviews, (1994) 46(1):89-109), however, bleeding and thrombocytopenia concerns have not been eliminated. A major effort has been devoted to the synthesis of the minimal ATIII binding pentasaccharide of heparin, and various sequence analogs (van Boeckel and Petitou, Ang. Chem. (1993), 32(12); 1671-1818) to provide single chemical entities for therapeutic development. This approach has provided oligosaccharide based sequences that are very potent anticoagulant and antithrombotic drug candidates. However, the practicality of commercial synthesis for these oligosaccharides, even though simplified relative to the natural pentasaccharide, and the lack of oral bioavailability, remain major unaddressed issues.

Thus, non-glycosidic heparin sequence mimetics of the ATIII-binding pentasaccharide have the potential to yield improved antithrombotic therapeutics. The natural

and synthetic AT III-binding pentasaccharide is shown below.

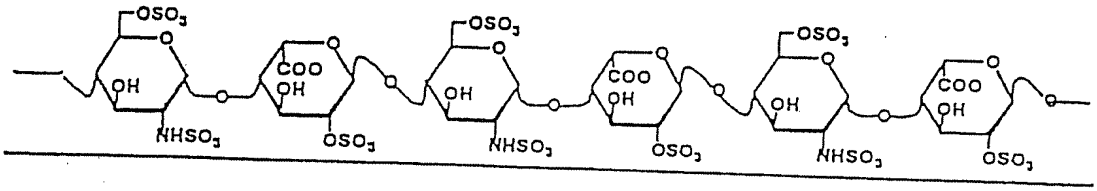


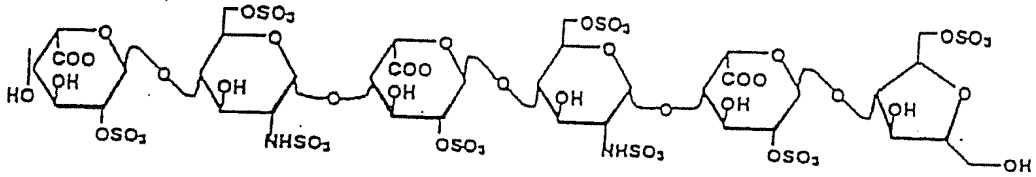


bFGF Antagonism Overview

The interaction of Heparin Binding Growth Factors (HBGF), such as bFGF (basic Fibroblast Growth Factor), with heparan sulfate and heparin are known to be required for mediation of the mitogenic activity of these proteins (A.O.M. Wilkie et al , Current Biology, (1995), (5(5); 500-506, M. Ishihara, Trends in Glycoscience and Glycotechnology, (1993), 5(25); 343-354). It has been established that the interaction of bFGF with heparan sulfate and heparin is mediated through a hexasaccharide sequence (D.J. Tyrrell et al , J. Biol. Chem. (1993) 268(7); 4684-4689, M. Maccarana, B. Casu and U. Lindahl, J. Biol. Chem. (1993), 268(32); 23898-23905) and that such hexasaccharides when isolated retain the bFGF antagonist properties, and lose the bFGF stimulatory properties associated with polymeric heparan sulfate. (M. Ishihara, Anal. Biochem (1992) 202; 310-315., M. Ishihara et al, Glycobiology (1994) 4(4): 451-458). The natural and

isolated bFGF binding hexasaccharide sequences are shown below.

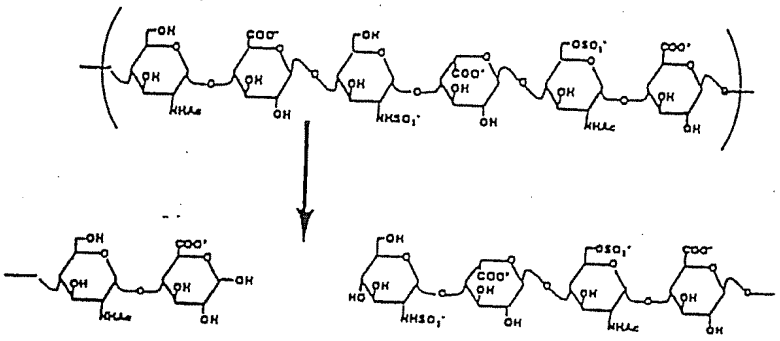




Heparanase Inhibition Overview

The heparanases, a family of endoglycosidases which hydrolyze internal glycosidic linkages of heparan sulfate ("HS") and heparin chains, can be found in a variety of tissues, and in normal and malignant blood-borne cells (Nakajima *et al.*, J. Cell Biochem. (1988) 36:157; Vlodavsky *et al.*, Invasion Metastasis (1992) 12:112). These enzymes have been postulated to participate in new blood vessel formation (angiogenesis) by releasing heparin-binding growth factors such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) from the extracellular matrix (ECM) (Bashkin *et al.*, Biochem. (1989) 28:1737) and to complement the activities of serine proteases and matrix metalloproteases in remodeling subendothelial basement membrane and ECM (Blood and Zetter, Biochim. Biophys. Acta. (1990) 1032:89). It has also been postulated that metastatic tumor cells egress from the vasculature by utilizing

matrix-degrading enzymes (endoglycosidases and proteases) to invade subendothelial basement membrane, ECM and interstitial stroma (Liotta *et al.*, Cell (1991) 64:327). This invasive phenotype has been shown to correlate with increased levels of heparanase activity in a number of metastatic tumor cell variants (Nakajima *et al.*, J. Cell. Biochem. (1988) 36:157). It has also been suggested that endothelial heparanase plays a role in atherosclerosis, Sivavam, *et al.* J. Biol. Chem. (1995) 270(50):29760-29765. Thus, heparanase inhibitors may be used to prevent or treat atherosclerosis.



Heparin has been shown to exhibit heparanase-inhibitory activity *in vitro* (Irimura *et al.*, Biochem. (1986) 25:5322, to inhibit endothelial cell (EC) ECM HSPG (heparan sulfate proteoglycan) degradation (Bar-Ner *et al.*, Int. J. Cancer (1987) 40:511); Parish *et al.*, 1987), and to block the dissemination of metastatic tumor cells to the lungs (Irimura *et al.*, (1986) supra. Heparin can be administered only at relatively low concentration *in vivo* due to its anticoagulant potency and potential for inducing hemorrhagic complications (Levine *et al.*, (1989) "Heparin-induced Bleeding" in Heparin, Lane and Lindahl (eds), CRC Press Inc., Boca Raton, FL, pp. 517-531). To enable the administration of drug at higher concentrations

without risking excessive bleeding associated with heparin administration, it would be extremely valuable to identify small molecule mimics of the minimal enzyme-heparan sulfate binding sequence. Typically, heparanase inhibitory activity has been shown to reside in modified heparin polymers and in oligosaccharides derived from heparin. Recently, it has been reported that siastatin, a known neuraminidase inhibitor, and related structures can inhibit the activity of heparanase derived from the murine B16-F10 metastatic cancer cell line , (Y. Kumase et al., J. Antibiotics, (1996), 49(1) 54 - 60, and 61-64).

Heparanases have been implicated in tumor cell proliferation, metastasis, and tumor neovascularization. A quantitative assay for heparanase was developed to assess which chemical groups in heparin, a well-documented "inhibitor" of heparanases (Nakajima *et al.*, 1988), might contribute to heparanase-inhibitory activity. Such information may lead to the development of better heparanase inhibitors. Saccharopeptides of the present invention are glycomimetics that may act as inhibitors of heparanase as the siastatin-like compounds have been shown to do.

The major road block in the discovery and development of heparin-derived drugs has been the prohibitive cost an difficulty in either the isolation or synthesis of the actual natural sequences. Various approaches from total synthesis of representative oligosaccharides and analogs (Van Boeckel *et al.*, Ang. Chem (1993) 32 (12); 1671-1818, M. Nilsson, Carbohydr. Res. (1993) 246: 161-172., T.M. Slaghek et al, Tetrahedron: Asym. (1994) 5(11): 2291-2301.) to the

modification and sulfation of readily available oligosaccharides (H. Wessel et al, J. Carbohyr. Chem (1996) 15(2): 201-216, H. Wessel et al, Bioorg. Med. Chem Lett. (1996) 6(4): 427-430) have been attempted. As yet, no practical approach for synthesizing non-glycoidic heparan sulfate sequence mimics has been described.

Saccharopeptides as described herein offer a convenient, practical approach to diverse "oligosaccharopeptides" suitable for discovery and development of heparan sulfate sequence glycomimetics useful for many of the disease areas where heparin has been beneficially implicated. The interactions of heparin with other proteins are under intensive study, and it is clear that specificity differences are possible and that these interactions have the potential to provide additional drug discovery targets (A.D. Lander, Chemistry and Biology, (1994)1; 73-78, D.P. Witt and A.D. Lander, Current Biology (1994) 4(5), 394-400.).

Anti-Infectives

Carbohydrates are known to be important cell surface ligands for viral adhesion processes. By targeting the inhibition of enzymes (glycosides) involved in the processing of viral glycoproteins, carbohydrates can act as anti-viral agents. For example, Deoxynojirimicin and costano-spermine inhibit viral infectivity presumably due to their glucosidease inhibitory activity. (R.A. Gruten et al., Nature (1987) 330:74; and D.D. Walden et al., Proc. Natl. Acad. Sci., USA (1987) 84:8120; and L. Ratner, Aides Research and Human Retroviruses (1991) 8(2):165 173.) Similarly, inhibitors of the enzyme Neuraminidase

(α-Sialidase) have also been show to inhibit the *in vitro* infectivity of influenza virus (J.O. Nagy et al. J. Med. Chem. (1992) 35:4501-4502) and are currently undergoing clinical evaluation. (I.D. Stanley et al. , Tet. Lett. (1995) 36(2):299-302; and M. von-Itastein et al., Nature (1993) 363:418-423)

A second carbohydrate based mechanism suitable as a drug development approach involves inhibitors of viral binding. It is believed that heparan sulfate supports the binding of HIV, and it is possible that heparin sequence mimics may function as inhibitors of viral binding. In both carbohydrate-based anti-viral strategies of drug development, the ability to generate novel glycomimetic compounds would be great advantage.

Carbohydrates as Anti-bacterial Targets

Antibiotics are the most widely employed class of carbohydrate therapeutics, with streptomycin as the first example (S. Umesawa, Adv. Carbohydr. Chem. Biochem. (1974) 30:111). Several classes of carbohydrate related antibiotics have been discovered ranging from glycopeptides, to macrocyclic compounds, and more recently to synthetic glycomimetics. In general, the carbohydrate component seems to be required for optimal activity (G. Lukacs, Recent Progress in the Chemical Synthesis of Antibiotics and Related Microbial Products, Springer-Verlay New York 1993). The saccharopeptides of the present invention may possess anti-bacterial activity.

Glycomimetic Approaches for Immunomodulation

A number of carbohydrates containing molecules, particularly glycopeptides such as, muramyl-dipeptide, and analogs, and glycolipids such as Lipid A, are known to modulate immune responses. (O. Lockoff, Angew Chem. Int. Ed. Engl. (1991) 30: 1611-1620.) and J.H. Musser et al. *Burgers Medicinal Chemistry and Drug Discovery, Fifth Ed., Volume 1; Principles and Practice*, Ed. Manfred E. Wolff, 1995 John Wiley and Sons Inc., pp 901-947). The glycomimetics of the present invention may act as immunomodulators.

The main limitations associated with many of the carbohydrate derived therapeutics is the presence of relatively unstable O-glycosidic linkages. This is an important factor causing often poor *in vivo* stability or non-optimal pharmacodynamic properties associated with carbohydrate containing therapeutics. Natural carbohydrates are very complex in structure and are both challenging and expensive to isolate or synthesize. The chemical synthesis of oligosaccharides requires sophisticated strategies that control product stereochemistry and regiochemistry. Enzymatic synthesis using glycosyltransferases and glycosidases is a viable alternative for ligands closely related to natural substances but is limited by the availability of enzymes with appropriate specificities.

Chemical Synthesitic Overview

The complex nature of carbohydrates and the relatively difficult synthetic procedures associated with the assembly of complex carbohydrates has limited the

application of medicinal chemistry to optimizing the therapeutic potential of carbohydrate therapeutics. Some approaches directed toward stabilized carbohydrates and other glycomimetic structures have been pursued in recent years, in part to address this need. For example, heteroatom glycosides, particularly S- and C-glycosides have become popular substitutes for O-glycosides, and have provided avenues into stabilized carbohydrate-like structures. While these approaches offer some potential, in reality, depending on the specific target of interest, heteroatom glycosides can present an even greater synthetic challenge than that of the natural carbohydrate structure, including control of stereochemistry. This lack of stereoselectivity presents a major obstacle in generating oligosaccharide combinatorial libraries.

This invention describes a general methodology based on relatively simple synthetic avenues for generating diverse, novel glycomimetic structures. This approach is suitable for discreet chemical synthesis of glycomimetic molecules or the generation of glycomimetic combinatorial libraries, offering significant advancement in the practicality of the discovery and development of glycomimetic therapeutics. This invention also provides a generalized approach, whereby carbohydrate mediated specificity can be combined with considerable spatial and functional diversity to generate glycomimetic structures with the potential to provide therapeutic activity via carbohydrate-mediated interactions, as discussed above.

There are scattered examples of synthetic compounds in the literature where monosaccharides are linked to each other by linkages other than the glycosidic bond. These

include examples of disulphide (Whistler, R.L., *et al.*, J. Org. Chem. (1964) 29:1259), hydrazine (Freudenberg, K., *et al.*, Ber Dtsch. Chem. Ges. (1925) 58:294), carbodiimide (Kovacs, J., *et al.*, Carbohydr. Res. (1987) 166:101), carbamide (Jones, A. S., *et al.*, Tetrahedron (1962) 18:189), and thiocarbamide (Avalos, M., *et al.*, J. Chem. Soc. Perkin Trans. I (1990) 495, and references therein) bridges. However, these examples are limited to pseudo-disaccharides, and in most cases the chemistry used for the preparation of these compounds excludes the synthesis of higher homologs.

Yoshimura, *et al.* prepared certain amido-bonded disaccharides containing hexosaminuronic acids (Yoshimura, J. *et al.* Bull. Chem. Soc. Jap. (1976) 49(a):2511-2514.) In contrast to the present invention, Yoshimura, *et al.* only prepared neutral disaccharides without a carboxylic acid group on a terminal sugar. Yoshimura, *et al.* attempted, but failed to make anything larger than an amido-linked disaccharide.

Recently some researchers have reported the synthesis of carbohydrate containing peptides. Von Roedern, *et al.* prepared cyclic somatostatin analogues and a linear leucine-eukephalin analogue containing the sugar amino acid glucosyluronic acid methylamine. They described this sugar amino acid as a dipeptide isostere. (Von Roedern, E.G. and Kessler, H., Angew Chem. Int. Ed. Engl, (1994) 33:687; Von Roedern, E.G. and Kessler, H., Abstract No. A1.12, XVIIth International Carbohydrate Symposium, Ottawa, July 17-22, 1994.) Wittman, *et al.* prepared C- and S-glycosylated alanine derivatives of the decapeptide gonadotropin releasing hormone to increase

water solubility and increase metabolic stability. Wittmann, V., *et al.*, Abstract No. C2.32, XVIIth International Carbohydrate Symposium, Ottawa, July 17-22, 1994. However, these examples are limited to modification of the peptide backbone, either to conformationally restrict the peptide, or to increase the metabolic stability of the peptides. There is no showing of saccharides linked to each other via a peptidic linkage in these examples.

PCT Publication WO95/04751 describes glycopeptides that have a three-dimensionally stable configuration and include fucose covalently linked to an amino acid or a peptide with a free carboxylic acid group. Such compounds were designed to bind selectins. These structures are O-glycosides attached to L-serine or L-threonine in the peptide backbone. There is no showing of saccharides linked to each other via a peptide linkage.

United States Patent No. 5,008,247 describes polysulfuric acid esters of aldonamides. It is important to note that in these examples the bis aldonic acid derivatives are limited to the open chain form of the carbohydrate moiety.

The present invention relates to a novel class of carbohydrate derivatives, saccharopeptides, which contain a carbohydrate derivative, analogue, or mimic linked to each other or to a peptide or other type of compound via a peptide bond, and to methods for using these saccharopeptides including purification of proteins using affinity chromatography, and as drugs, to treat certain diseases.

Summary of the Invention

A first object of the invention is the description of novel compounds that contain a carbohydrate derivative bound to another carbohydrate or other group through a peptide bond. Such compounds mimic natural carbohydrate ligands. Such inventive compounds are represented by the following general structural formula I:

$$W-(X)_n-Y-[(X)_n-W-(X)_n-Y]_m-(X)_n-W \qquad (I)$$

wherein

W is independently selected from the group consisting of

a) saccharides;

b) aryl, aralkyl;

c) alkyl of 1 to 8 carbon atoms, optionally substituted with 1-2 substituents selected from the group consisting of lower aryl, lower alkyl, =O, -OR, $-NR'_2$, -SR, $-SO_4R$, $-SO_3R$, -COOR, -alk-COOR; and

d) cyclic alkyl or 5-7 carbon atoms, heterocyclic alkyl of 5-7 ring atoms and 1-2 heteroatoms selected from the group consisting of N, O, and S, all optionally substituted with 1-5 substituents selected from the group consisting of =O, -OH, -OR, $-NR'_2$, -SR, $-SO_4R$, $-SO_3R$, -COOR, and -alk-COOR;

Y is independently selected from the group consisting of $-NR^3-C(O)-$ and $-C(O)-NR^3-$;

X is a difunctional or polyfunctional group selected from the group consisting of

a) aryl, aralkyl;

b) alkyl of 1-8 carbon atoms, optionally substituted with 1-3 substituents in the alkyl backbone or exo to the backbone selected from the group consisting of lower aryl, lower alkyl, -O-, -NR'-, -S-, =O, -OH, -OR, -NR'_2, -SH, -SR, -SO_4R, -SO_3R, -COOR, and -alk-COOR;

each n is independently 0 or 1;

m is independently 0 or an integer from 1 to 99 with the proviso that the total number of W groups is 2-100;

R is -H, or lower alkyl, lower aryl, and lower aralkyl;

R'is independently selected from the group consisting -H, lower alkyl of 1-4 carbon atoms, aralkyl of 2 to 19 carbon atoms, and -C(O)R";

R" is lower alkyl of 1 to 4 carbon atoms; and

R^3 is selected from the group consisting of -H, alkyl of 1-8 carbon atoms, and aralkyl of 5-8 carbon atoms;

and pharmaceutically acceptable salts thereof; with the following provisos:

a) when the total number of W groups is 2, then both W groups may not be 2-amino hexoses;

b) at least one W group is a saccharide; and

c) if the terminal W is a N-acetylglucosamine, it may not be linked through -NHCO- at the anomeric carbon to a natural amino acid.

A second object of the invention is a description of saccharopeptides that can be used in the treatment or prevention of certain diseases including cancer,

cardiovascular disease, retinopathies, inflammation, auto-immunity, and bacterial and viral infections.

A third object of the invention is a description of saccharopeptides that inhibit cell-cell adhesion.

A fourth object of the invention is to provide saccharopeptides that bind to selectins.

A fifth object of the invention is to provide saccharopeptides that inhibit angiogenesis.

A sixth object of the invention is to provide saccharopeptides that inhibit the binding of proteins to heparin.

A seventh object of the invention is to provide saccharopeptides that inhibit the binding of bFGF.

An eighth object of the invention is to provide saccharopeptides that can inhibit carbohydrate processing enzymes, both anabolic and catabolic.

A ninth object of the invention is to provide saccharopeptides that inhibit heparanase.

A tenth object of the invention is to provide saccharopeptides that can be used for purification of proteins using affinity chromatography.

These and other objects, advantages, and features of the present invention will become apparent to those persons skilled in the art upon reading the details of the synthesis, structure, formulation and usage as more fully set forth below.

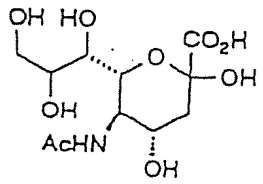
Definitions

In accordance with the present invention and as used herein, the following terms are defined with the following meanings, unless explicitly stated otherwise.

"Sialic acids" refer to the family of amino sugars containing 9 or more carbon atoms, N- and O- substituted derivatives of neuraminic acid.

"Kemp's acid" refers to 1,3,5-trimethyl-1,3,5-cyclohexane-tricarboxylic acid, where each acid is axial. It is a suitable sialic acid mimic.

"N-acetyl neuraminic acid" refers to 5-(acetylamino)-3,5-dideoxy-D-glycero-D-galacto-2-nonulosonic acid:



"β form" refers to standard nomenclature representing the configuration of the anomeric position of an O- or C-glycoside.

"α form" refers to standard nomenclature representing the configuration of the anomeric position of an O- or C-glycoside.

"Amino" refers to $-NR_2$ where each R is independently selected from -H, lower alkyl, lower aryl, and lower aralkyl.

"Alkyl" refers to saturated hydrocarbons, which may be straight chain, branched, cyclic, or alicyclic. Preferably the alkyl group contains 1 to 8 carbon atoms. Most preferred is 1 to 4 carbon atoms.

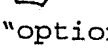
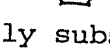
"Lower alkyl" refers to branched or straight chain alkyl of 1 to 4 carbon atoms.

"Alkoxy" refers to -OR, where R is an alkyl group. Lower alkoxy refers to -OR where R is lower alkyl.

"Aryl" refers to aromatic groups which have one to three rings having a conjugated pi electron system and includes carbocyclic aryl, and heterocyclic aryl, both of which may be optionally substituted. Suitable polycyclic aryl groups include napthyl, and anthracyl. Preferably, the aryl has 1-14 carbon atoms, and more preferably 4-14 carbon atoms. Lower aryl refers to an aryl containing up to 6 carbon atoms, and may be optionally substituted.

"Carbocyclic aryl" groups are groups wherein the ring atoms are carbon atoms.

"Heterocyclic aryl" groups are groups having from 1 to 4 heteroatoms in the ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include nitrogen, oxygen, and sulfur. Suitable heterocyclic aryl groups include pyridyl, furanyl, thienyl, pyrrolyl, triazolyl, tetrazolyl and the like all optionally substituted. Heteroaryl is the same as heterocyclic aryl.

"Alicyclic" refers to groups which combine the properties of aliphatic and cyclic alkyl groups. For example, $-CH_2$—  and  —CH_3 are alicyclic groups.

The term "optionally substituted" refers to either no substitution or substitution by one to three substituents independently selected from lower alkyl, -OH, -OR, -SR, -SH, $-NR'_2$, $-SO_4R$, $-SO_3R$, halo, carboxylic acids, esters, $-NO_2$, and lower perhaloalkyl, where R is H or lower alkyl, and R' is H, lower alkyl, aralkyl, and lower acyl.

"Aralkyl" refers to an alkyl group substituted with an aryl group, which may be optionally substituted. Benzyl is a suitable aralkyl group. Preferably, the aralkyl group has 2-19 carbon atoms, and more preferably 5-19 carbon atoms. Lower aralkyl refers to up to and including 8 carbon atoms, and may be optionally substituted. The aralkyl group is attached through the alkyl portion of the group.

"Alkenyl" refers to unsaturated groups which contain at least one carbon-carbon double bond and includes straight-chain, branched chain, and cyclic groups. The double bond may be exo to the chain.

"Alkoxyaryl" refers to aryl substituted with alkoxy group.

"Alkynyl" refers to unsaturated groups which contain at least one carbon triple bond and includes straight-chain, branched chain, and cyclic groups.

"Aryloxy" refers to -O-aryl.

"Aralkoxy" refers to -O-aralkyl.

"Carboxylic acid" refers to -COOH.

"Ester" refers to -COOR where R is lower alkyl, lower aryl, and lower aralkyl;

"Amide" refers to $-CONR_2$ where each R is independently selected from hydrogen, lower alkyl, lower aryl, and lower aralkyl. Preferably at least one R is hydrogen.

"Acyl" refers to -C(O)R, where R is alkyl, aralkyl, and aryl.

"Protecting group" refers to a group protecting one or several inherent functional groups. Suitable "protecting groups" will depend on the functionality and particular chemistry used to construct the compound or the library. Examples of suitable functional protecting groups will be readily apparent to skilled artisans, and are described, for example, in Greene and Wutz, Protecting Groups in Organic Synthesis, 2d ed., John Wiley & Sons, NY (1991), which is incorporated herein by reference.

Suitable -0-protecting groups can be found in the above book. Preferred such protecting groups include acetate, benzoyl, and benzyl.

"Saccharides" refer to carbohydrate derivatives having a chemical moiety comprising the general composition $(C)_n(H_2O)_n$, including, but not limited to glucose, galactose, fucose, fructose, saccharose, mannose, arabinose, xylose, sorbose, lactose, and derivatives, thereof, including but not limited to compounds which have other elemental compositions, such as aldonic acids, uronic acids, desoxysugars, or which contain additional elements or moieties, such as amino sugars wherein n is typically 4, 5, 6, 7 atoms and wherein the oxygen atom in the saccharide can be replaced by a heteroatom such as nitrogen, sulfur, and carbon etc. The term "saccharides" include carbon glycosides. A saccharide as used herein is understood to include chemical structures wherein "H" of any hydroxy group is replaced by any chemically compatible moiety "R", which can be any monomer, oligomer or polymer in the meaning as used herein. The

saccharide(s) can be in the pyranose or furanose ring form and have either an α or β configuration at the anomeric center. Oligomeric saccharides are independently covalently linked by an ether, thioether, glycosidic, thioglycosidic, carbon glycosidic, or amino bond.

The hydroxyl or amine groups of the saccharide are optionally replaced with H, halogen, -COOH or OR^1, where R^1 is an alkyl, aryl, aralkyl, acyl, all optionally substituted, protecting group, and lipids. Saccharides can be saturated or unsaturated. Saccharides may be charged or uncharged. Suitable charged saccharides include galacturonic acid, glucuronic acid, and sialic acid.

"Carbohydrate unit" is a monomer comprising a monosaccharide. Examples of suitable monosaccharides useful in the present invention include, but are not limited to, D-glucose, D-galactose, D-mannose, D-xylose, D- and L-arabinose, D-ribose, L-rhamnose, L-fucose, D-glucuronic acid, D-galacturonic acid, L-iduronic acid, D-glucosamine, D-galactosamine, D-lyxosamine, glucosamine uronic acid and sialic acid.

"Oligomers" and "oligosaccharides" refers to carbohydrates including carbon glycosides, comprising a plurality of monosaccharides. This includes disaccharides, trisaccharides, etc., and preferably 3-12 monomer units. Examples of disaccharides useful in the present invention include, but are not limited to maltose, lactose, cellobiose, melibiose and 3-O-β-D-galactopyranosyl-D-arabinose. Examples of trisaccharides and higher oligosaccharides useful in the

present invention include, but are not limited to, maltotriose, and maltotetraose.

"Non-saccharide" refers to W groups selected from the group consisting of

a) aryl, aralkyl;

b) alkyl of 1 to 8 carbon atoms, optionally substituted with 1-2 substituents selected from the group consisting of lower aryl, lower alkyl, =O, -OH, -OR, -NR'$_2$, -SH, -SR, -SO$_4$R, -SO$_3$R, -COOR, -alk-COOR; and

c) cyclic alkyl or 5-7 carbon atoms, heterocyclic alkyl of 5-7 ring atoms and 1-2 heteroatoms selected from the group consisting of N, O, and S, all optionally substituted with 1-5 substituents selected from the group consisting of =O, -OH, -OR, -NR'$_2$, -SR, -SO$_4$R, -SO$_3$R, -COOR, and -alk-COOR;

"Difunctional" or "polyfunctional" alkyl, aryl or aralkyl group, amino acid or peptide refers to groups capable of linking two saccharide units together through preferably either an ether, thioether, glycosidic, thioglycosidic, amino or amido bond. Examples include but are not limited to diols, oligomers of diols, aromatic diols such as hydroquinone and dihydroxynaphthalenes, aralkyl diols such as benzenedimethanol, dithiols, oligomers of dithiols and thiohydroxy compounds, diamines, oligomers of diamines, dicarboxylic acids and oligomers of dicarboxylic acids. Optionally, the group may possess additional functional groups such as hydroxyls, thiols, amines, carboxylic acids, amides or sulfonic acids wherein these groups do not form bonds with the saccharide units.

"Carbon glycoside" is a carbohydrate derivative wherein the anomeric position does not have an oxygen but a carbon substituent.

"Peptide" bond or link refers to -NR-C(O)- and -C(O)-NR- where R is hydrogen, alkyl, aryl, or aralkyl.

"Heteroatom glycoside" is a carbohydrate wherein the oxygen at the anomeric position is replaced by an atom other than oxygen, including carbon, nitrogen, sulfur, phosphorous and silicon.

"Identifier tag" is any detectable attribute that provides a means to elucidate the structure of an individual oligomer in a labeled synthetic oligomer library. For example, an identifier tag can be used to identify the resulting products in the synthesis of a labeled synthetic oligomer library.

"Named Reactions" are chemical reactions which are chemical standard reactions known by those of ordinary skill in the art, including but not limited to the Alper Reaction, Barbier Reaction, Claisen-Ireland Reaction, Cope Rearrangement, Delepine Amine synthesis, Gewald Heterocycle Synthesis, Hiyama-Heathcock Stereoselective Allylation, Stork Radical Cyclization, Trost Cyclopentanation, Weidenhagen Imidazole Synthesis. *See*, in general, Hassner and Stumer, 1994. *See*, among other places, "Organic Syntheses Based on Named Reactions and Unnamed Reactions", Tetrahedron Organic Chemistry Series, edts. Baldwin and Magnus, Pergamon, Great Britain.

"Polysaccharide" refers to carbohydrates, including carbon glycosides, comprising a plurality of monosaccharides.

"Synthetic chemical library" is a collection of random and semi-random synthetic molecules wherein each member of such library is produced by chemical or enzymatic synthesis.

A "Synthesis support" is a material having a rigid or semi-rigid surface and having functional groups or linkers. A synthesis support may be capable of being derivatized with functional groups or linkers that are suitable for carrying out synthesis reactions. Such materials will preferably take the form of small beads, pellets, disks, capillaries, hollow fibers, needles, solid fibers, cellulose beads, pore-glass beads, silica gels, polystyrene beads optionally cross-linked with polyethylene glycol divinylbenzene, grafted co-poly beads, poly-acrylamide beads, latex beads, dimethylacrylamide beads optionally cross-linked with N,N'-bis-acryloyl ethylene diamine, glass particles coated with a hydrophobic polymer, or other convenient forms.

"Transformation event" or "Reaction" is any event that results in a change of chemical structure of a compound, monomer, an oligomer or polymer. A "transformation event" or "reaction" may be mediated by physical, chemical, enzymatic, biological or other means, or a combination of means, including but not limited to, photo, chemical, enzymatic or biologically mediated isomerization or cleavage, photo, chemical, enzymatic or biologically mediated side group or functional group addition, removal or modification, changes in temperature, changes in pressure, and the like. Thus, "transformation event" or "reaction"

includes, but is not limited to, events that result in an increase in molecular weight of a monomer, an oligomer or polymer, such as, for example, addition of one or a plurality of monomers, addition of solvent or gas, or coordination of metal or other inorganic substrates such as, for example, zeolites. A "transformation event" or "reaction" may also result in a decrease in molecular weight of an oligomer or polymer, such as, for example, de-hydrogenation of an alcohol to form an alkene or enzymatic hydrolysis of an ester or amide. "Transformation events" or "reaction" also include events that result in no net change in molecular weight of a monomer, an oligomer or polymer, such as, for example, stereochemistry changes at one or a plurality of a chiral centers, Claissen rearrangement, Ireland rearrangement, or Cope rearrangement and other events as will become apparent to those skilled in the art upon review of this disclosure.

"Heterocyclic alkyl" refers to a cyclic alkyl group in which one to three of the ring atoms are a heteroatom and the remaining ring atoms are carbon atoms. Suitable heteroatoms are nitrogen, oxygen, and sulfur. Suitable heterocyclic alkyl groups are morpholine, piperadine, and piperazine.

"-Ar-" refers to a phenyl, optionally substituted.

"-alk-" refers to an alkyl linking group which is selected from lower alkyl, and cycloalkyl. Suitable "-alk-" groups include $-C(CH_3)_2-$, and .

"Halo" refers to halogen atoms -F, -Cl, -Br, and -I.

"Cycloalkyl" refers to cyclic alkyl groups and include cyclopropyl, cyclopentyl, cyclohexyl, and cycloheptyl.

The term "pharmaceutically acceptable salt" includes salts of compounds of formula I derived from the combination of a compound of this invention and an organic or inorganic acid or base. The compounds of formula I are useful in both the free acid, free base, and salt form.

Detailed Description of the Invention

Throughout the description of the invention reference is made to certain publications including scientific articles and patents or patent applications. It is the intent that each of these publications be incorporated by reference in their entirety when referred to in the specification.

Before describing the present invention it is to be understood that this invention is not limited to the particular compositions, methods or processes described as such compositions and methods may, of course, vary.

As used in this specification and the appended claims, the singular forms "a", "an" and "the" include the plural unless the context clearly dictates otherwise. Thus, for example, reference to "an E-selectin", "a P-selectin", or "an L-selectin" includes reference to respective mixtures of such molecules, reference to "the formulation" or "the method" includes one or more formulations, methods and/or steps of the type described herein and/or which will become apparent

to those persons skilled in the art upon reading this disclosure.

Some standard abbreviations used in connection with the present invention include: BSA, bovine serum albumin; DEAE,-diethylaminoethyl; DMSO, dimethylsulfoxide; DMF, N,N-dimethylforamide; DCE, dichloroethane; E-selectin or ELAM-1, endothelial/leukocyte adhesion molecule-1; HPTLC, high performance thin layer chromatography; L-selectin or LECAM-1, leukocyte/endothelial cell adhesion molecule-1; MOPS, 3-[N-Morpholino) propanesulfonic acid; NANA, N-acetylneuraminic acid; PVC, polyvinylchloride; TLC, thin layer chromatography; TFA, trifluoro-acetic acid; Tris, tris (hydroxy-methyl) aminomethane.

The novel saccharopeptide compounds of the present invention are designed to be glycomimetics without all of the synthetic challenges inherent in full glycosidically linked compounds. These novel saccharopeptides are represented by the following general structural formula I:

$$W\text{-}(X)_n\text{-}Y\text{-}[(X)_n\text{-}W\text{-}(X)_n\text{-}Y]_m\text{-}(X)_n\text{-}W \qquad (I)$$

wherein

W is independently selected from the group consisting of

a) saccharides;

b) aryl, aralkyl;

c) alkyl of 1 to 8 carbon atoms, optionally substituted with 1-2 substituents selected from the

group consisting of lower aryl, lower alkyl, =O, -OR, $-NR'_2$, -SR, $-SO_4R$, $-SO_3R$, -COOR, -alk-COOR; and

d) cyclic alkyl or 5-7 carbon atoms, heterocyclic alkyl of 5-7 ring atoms and 1-2 heteroatoms selected from the group consisting of N, O, and S, all optionally substituted with 1-5 substituents selected from the group consisting of =O, -OH, -OR, $-NR'_2$, -SR, $-SO_4R$, $-SO_3R$, -COOR, and -alk-COOR;

Y is independently selected from the group consisting of $-NR^3-C(O)-$ and $-C(O)-NR^3-$;

X is a difunctional or polyfunctional group selected from the group consisting of

a) aryl, aralkyl;

b) alkyl of 1-8 carbon atoms, optionally substituted with 1-3 substituents in the alkyl backbone or exo to the backbone selected from the group consisting of lower aryl, lower alkyl, -O-, -NR'-, -S-, =O, -OH, -OR, $-NR'_2$, -SH, -SR, $-SO_4R$, $-SO_3R$, -COOR, and -alk-COOR;

each n is independently 0 or 1;

each m is independently 0 or an integer from 1 to 99 with the proviso that the total number of W groups is 2-100;

R is -H, or lower alkyl, lower aryl, and lower aralkyl;

R'is independently selected from the group consisting -H, lower alkyl of 1-4 carbon atoms, aralkyl of 2 to 19 carbon atoms, and -C(O)R";

R" is lower alkyl of 1 to 4 carbon atoms; and

R^3 is selected from the group consisting of -H, alkyl of 1-8 carbon atoms, and aralkyl of 5-8 carbon atoms;

and pharmaceutically acceptable salts thereof; with the following provisos:

a) when the total number of W groups is 2, then both W groups may not be 2-amino hexoses;

b) at least one W group is a saccharide; and

c) if the terminal W is a N-acetylglucosamine, it may not be linked through -NHCO- at the anomeric carbon to a natural amino acid.

One preferred aspect of the present invention is compounds of formula I

wherein m is an integer of 1 to 99. Preferred are such compounds wherein m is an integer of 1-5; and W is independently selected from the group consisting of fucose, 3-amino-3-deoxyglucose, 4-amino-4-deoxy-glucose, glucose, galactose, glucosamine, galactosamine, glucuronic acid, galacturonic acid, glucosamine uronic acid, neuraminic acid, maltose, maltotriose iduronic acid, 2,5-anhydromannitol, mannose, mannuronic acid, and cellobiose. Particularly preferred are such compounds wherein m is an integer of 1-2; and W is independently selected from glucuronic acid, and glucosamine.

Another preferred group of compounds are those wherein W is selected from the group consisting of maltose, maltotriose, and cellobiose. Such compounds where n and m are 1; and X is independently selected from the group consisting of ethylene glycol, ethylene glycol oligomers, lower alkyl, optionally subsituted alkyl, amino acid, and peptides.

Another preferred aspect of the present invention is compounds having the formula

W' - Y - W" - Y - W'

wherein

each W' is independently selected from the group consisting of saccharides;

W" is selected from the group consisting of

a) aryl, aralkyl,

b) alkyl of 1 to 8 carbon atoms, optionally substituted with 1 to 2 substituents selected from the group consisting of lower aryl, lower alkyl, =O, -OR, $-NR'_2$, -SR, $-SO_4R$, $-SO_3R$, -COOR, -alk-COOR; and

c) cyclic alkyl of 5-7 carbon atoms, heterocyclic alkyl of 5-7 ring atoms and 1-2 heteroatoms selected from the group consisting of N, O, and S, all optionally substituted with 1-5 substituents selected from the group consisting of =O, -OH, -OR, $-NR'_2$, -SR, $-SO_4R$, $-SO_3R$, -COOR, and -alk-COOR; and

Y is -NH-CO-.

Another preferred aspect of the present invention is compounds having the formula

W' - Y - W' - Y - W"

wherein W', W", and Y are as defined above.

Another preferred aspect are compounds of formula I wherein at least one terminal W group is substituted with $-NR'_2$, SO_3R, or -COOR.

Preferred are compounds of formula I wherein the total number of W groups is 2-8. More preferred are such compounds wherein the total number of W groups is 3-4.

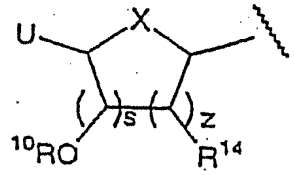
Another preferred aspect of the invention is compounds having the formula

$$W^1 - Y - W - Y - W^2$$

wherein

W^1 is selected from the group consisting of $-(C=O)R^{11}$, sialic acid, Kemp's acid, -B, $-SO_3M$, $-OSO_3M$, $-SO_2NH_2$, $-PO_3M'_2$, $-OPO_3M'_2$, $-NO_2$, saturated or unsaturated carboxylic acids of 1 to 4 carbon atoms, optionally substituted with 1 to 2 hydroxyl groups, and esters, and amides thereof;

W^2 is



wherein

U is selected from the group consisting of $-R^9$, $-CH_2OR^{10}$, $-CH_2O$-protecting group, $-COOR^{11}$, $-CON(R^{11})_2$, and -COOM;

R^9 is lower alkyl;

each s is independently selected from the group 1, 2, and 3;

each z is independently selected from the group 1 and 2;

R^{10} is selected from the group consisting of -H, $-R^{11}$, $-SO_3M$, $-(C=O)R^{11}$, $-SO_2NH_2$, $-PO_3M'_2$, -alk-$COOR_{13}$, -alk-$CON(R^{11})_2$ and -O-carbohydrate;

R^{11} is independently selected from the group consisting of -H, lower alkyl, cyclic alkyl of 5 to 6 carbon atoms, heterocyclic alkyl of 4 to 5 carbon atoms and 1 to 2 heteroatoms, lower aryl and lower aralkyl;

R^{13} is selected from the group consiting of R^{11}, and M;

R^{14} is selected from the group consisting of -H, and $-OR^{10}$;

M is selected from the group consisting of Na^+, K^+, Mg^{2+}, and Ca^{2+};

M' is selected from the group consisting of -H, -M, and R^9; and

X is selected from the group consisting of -O-, -S-, $-N(R^{11})-C(R^{11})_2-$, and $-N(R^{11})-$; and

B is a W^2 group containing at least one $-COOR^{11}$, $-CON(R^{11})_2$, -COOM, $-SO_3M$, or $-(C=O)R^{11}_2$ substituent.

Another preferred group of compounds of formula I are those having a total of 4-8 W groups, where 2-4 of said W groups are saccharides, optionally fully or partially sulfated.

Preferred are compounds having an Selectin ELISA IC_{50} of $\leq$ 250 μM. More preferred are compounds having an IC_{50} of $\leq$ 100 μM.

Preferred are compounds having an IC_{50} of < 10μg in the bFGF assay.

Administration and Use

The novel saccharopeptides of this invention may generate glycomimetics based on known carbohydrate therapeutics including: (1) carbohydrate-containing antibiotics; (2) glycosidase inhibitor based antiviral and anti-tumor agents; (3) adriamycin derived anticancer agents; (4) cardiac glycosides; and (5) heparin-derived pentasaccharide antithrombotics. More importantly, the diversity of glycomimetic compound generation offered by this invention will facilitate the discovery and development of new therapeutics based on carbohydrate interactions.

Some examples, which are described in greater detail herein, include: (1) selectin antagonists for inflammation and metastasis; (2) heparan sulfate sequence mimics as antagonists of heparan sulfate binding protein interaction, including antithrombotic activity mediated by antithrombin III interactions, cell proliferative activity of bFGF, the formation of amyloid plaque in Alzheimer's and other heparin binding growth factors associated with angiogenesis and cancer tumor growth; (3) inhibitors of carbohydrate biosynthetic enzymes, such as the glycosyltransferases (xylosides, fucosyltransferase inhibitors) that biosynthesize the complex carbohydrate epitopes that mediate important cellular recognition processes (J.H. Musser, *et al.*, "Carbohydrate Based Therapeutics in Medicinal Chemistry and Drug Discovery, Fifth Ed., Vol. 1, Ed. Manfred E. Wolff, 1995, John Wiley and Sons, pp. 901-947; and K.A. Karlsson, Glycobiology: A Growing Field for Drug Design, TiPS, July 1991 12:265-273); inhibition of glycation

reactions may prevent or treat Alzheimer's disease (Harrington, *et al.* Nature (1994) 370:247); and (4) inhibitors of carbohydrate degrading enzymes such as heparanases, which degrade extracellular matrix to facilitate extravasation of cancer and inflammatory cells through the vascular wall and tissue, and in the formation of atherosclerotic plaque.

The saccharopeptides of the instant invention are useful as inhibitors of β-glucuronidase. β-Glucuronidase is one of the most important enzymes involved in carbohydrate metabolism, and is widespread in mammalian tissues and body fluids, as well as in lower bacteria. Synthetic inhibitors of this enzyme aid in metabolic studies, along with providing a useful tool for purifying the enzyme by affinity chromatography (Y.C. Lee, *et al.*; Carbohydrate Research, (1978) 64:302). However, very few inhibitors of β-glucuronidase have been reported. The saccharopeptides of the instant invention are inhibitors of -β glucuronidase, and are useful in the purification of the enzyme by affinity chromatography.

Generally, enzyme activity is determined by measuring adsorption value of various aglycons (*e.g.*, phenolphthalein), which are liberated from glucuronic acid by the action of β-glucuronidase during the reaction.

The β-glucuronidase inhibition assay is preferably performed in a solvent. Examples of the solvent include water and a suitable buffer, preferably an acetate or an AMP (2-amino-2-methyl-1-propanol) buffer. Phenolphthalein mono-β-glucuronic acid and a

saccharopeptide substrate are treated with the enzyme at room temperature, at a pH of about 4-5. The reaction period ranges from 0.5-2 hours. After completion of the reaction, the enzymatic reaction is stopped by adjusting the pH and the absorbance is measured at 550 nm.

The saccharopeptides of the instant invention are also useful in therapeutic applications for treating or preventing a variety of diseases including cancer, auto-immune disorders, inflammation, infections, and diseases caused or exacerbated by platelet aggregation or angiogenic activity.

The cancers that can be treated include malignant tumors that are primary or metastatic, and benign tumors that do not metastasize, but may influence morbidity and mortality as a result of compression and/or obstruction due to shear mass. The following describes some of the cancers that may be treated, but is not intended to limit the scope of the invention. In the brain, Gioblastoma multiforme, Malignant Glioma, Medulloblastoma, Primary Lymphoma, and Meningioma may be treated. In the lung, Adenocarcinoma, Squamous Cell Carcinoma, Large Cell Undifferentiated Carcinoma, and Small Cell Carcinoma may be treated.

In the gastrointestinal tract, including the oral cavity and pharynx, esophagus, stomach, small intestine, large intestine, and anus, Squamous Cell Carcinoma, Adenocarcinoma, and Primary Lymphoma may be treated.

Liver cancer such as Heptocellular Carcinoma may be treated. Cancers of the Bone/Marrow that may be treated include Osteogenic Sarcoma, Multiple Myeloma, Leukemia

(erythro-, acute/chronic lymphoblastic-, acute/chronic myelogenous leukemias, and Ewing's Sarcoma.

In the breast, the cancers that may be treated include Ductal Adenocarcinoma, Lobular Carcinoma, Medullary Carcinoma, and Inflammatory Carcinoma.

In the Prostate/Bladder, the cancers that may be treated include Adenocarcinoma, Transitional Cell Carcinoma, and Squamous Cell Carcinoma. In the testes and ovaries, the cancers that may be treated include Adenocarcinoma (papillary/serous), Gonadal-Stromal Tumors, Teratocarcinoma, Embryonal Carcinoma, and Choriocarcinoma.

In the lymph nodes, the cancers that may be treated include Malignant Lymphomas, and Hodgkin's Lymphoma. Cancers of the skin, deep tissue, head, and neck that may be treated include Malignant Melanoma, Squamous Carcinoma, Basal-Cell carcinoma, Angiosarcoma, Kaposi's Sarcoma, Malignant Fibrous Histiocytoma, and Liposarcoma. In the uterus, the cancers and tumors that may be treated include Leiomyoma, Leiomyosarcoma, Adenosarcoma and Adenocarcinoma. Endocrine cancers may also be treated. This includes cancers of the pituitary, including Adenoma and Craniopharyngioma, cancers of the thyroid, including Papillary Carcinoma, Follicular Carcinoma, and Medullary Carcinoma, cancers of the pancreas, including Adenocarcinoma, and Islet Cell Carcinoma, and cancers of the adrenal gland including Adrenal Carcinoma. In the kidney, the cancers that may be treated include Renal Cell Carcinoma, and Wilm's Tumor. Pediatric tumors that are not necessarily specific to a single organ may also be treated. These include Neuroblastoma and Rhabdomyosarcoma.

Administration of the saccharopeptides of the invention can be by the normal routes, including but not limited to, intravenous, subcutaneous, oral, and inhalation. Preferred dosages for acute usage would be 0.1-10 mg/kg by iv. administration or 1-100 mg/kg/d by s.c. route. Preferred dosages for chronic disease by oral route or by inhalation would be 1-100 mg/kg/d. Typical dosage ranges are in the range of 0.1-100 mg/kg on a constant basis over a period of 5-30, preferably 7-14 days.

Injection subcutaneously at a lower dose or adminsitered orally at a slightly higher dose than intravenous injection, or by transmembrane or transdermal or other topical administration for localized injury may also be effective. Localized administration through a continuous release device, such as a supporting matrix, perhaps included in a vascular graft material, is particularly useful where the location of the trauma is accessible.

Formulations suitable for the foregoing modes of administration are known in the art, and a suitable compendium of formulations is found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA, latest edition.

The saccharopeptides may also be labeled using typical methods such as radiolabeling, fluorescent labeling, chromophores or enzymes, and used to assay the amount of such compounds in a biological sample following its administration. Conventional techniques for coupling of label to carbohydrates or related moieties can be used. Such techniques are well

establihsed in the art. See, for example, U.S. Patent No. 4,613,665. The labeled saccharopeptides may be used to identify sites of disease as well as in competitive immunoassays, and as a means to trace the pharmacokinetics of the compounds *in vivo*. Suitable radioisotope labels for this purpose include hydrogen^{3}, iodine^{131}, indium^{111}, technetium^{99}, and phosphorus^{32}. Suitable enzymic labels include alkaline phosphatase, glucose-6-phosphate-dehydrogenase, and horseradish peroxidase. Particularly preferred fluorescent labels include fluorescein and dansyl. A wide variety of labels of all three types is known in the art.

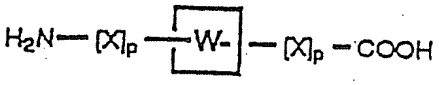
Suitable protocols for competitive assays of analytes in biological samples are well known in the art, and generally involve treatment of the sample, in admixture with the labeled competitor, with a specific binding partner which is reactive with the analyte such as, typically, an immunoglobulin or fragment thereof. The antibodies prepared according to the invention, as described below, are useful for this purpose. The binding of analyte and competitor to the antibody can be measured by removing the bound complex and assaying either the complex or the supernatant for the label. The separation can be made more facile by preliminary conjugation of the specific binding partner to a solid support. Such techniques are well known in the art, and the protocols available for such competitive assays are too numerous and too well known in the art, and the protocols available for such competitive assays are too numerous and too well known to be set forth in detail here.

General Synthesis

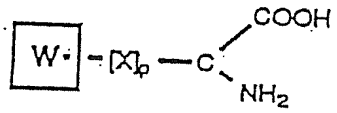
Attachment of the saccharides to each other by amide linkages can be achieved by the reaction of the amino and carboxylic acid groups, Y in formula I, wherein the amino and carboxylic acid groups, respectively, can be attached either directly, or through a X group, to the W group. Besides the amino and/or carboxylic acid substituents, the W groups may optionally be additionally substituted, with -H, halogen, -COOH, or $-OR^1$, where R^1 is an alkyl, aryl, aralkyl, acyl, optionally substituted, $-PO_3$, protecting group, and lipids. Further chain extension via peptide bonds is possible either with the above type carbohydrate derived amino acids (saccharo-amino acids), or with natural or non-natural amino acids.

Functionalization of monosaccharides to have the above two functional groups, giving the saccharo-amino acids, can be accomplished by using the standard methodologies of carbohydrate chemistry (Boger, J., *et al.*, Helvetica Chimica Acta, (1978) 61:2190, de Nooy, A.E.J., *et al.*, Carbohydrate Research, (1995) 269:89). Two types of saccharoamino acids are represented by the general formulae IV and V. In the saccharoamino acids of what hereinafter will be referred to as type A (formula IV), the amino and carboxylic groups are attached to different positions of the mono or oligosaccharide unit. Both functional groups can be attached directly the sugar ring, or alternatively, one or both of them can be linked to the sugar by a X group, wherein k is greater or equal to zero. In compounds of what hereinafter will be referred to as type B (formula

V) both the amino and the carboxylic groups are attached to the same carbon of the sugar ring, either directly or by a X group, wherein p is greater or equal to zero.



IV - Saccharo-amino acid (A)



V - Saccharo-amino acid (B)

Synthesis of saccharo-amino acids of either type A and/or B is achieved by converting a hydroxyl group to an amino group and another hydroxyl group to a carboxylic group.

Introduction of an amino group can be achieved by a variety of ways, such as

(a) converting the hydroxyl group into a leaving group, and further substituting the leaving group with an appropriate amino functionality. This approach involves conversion of hydroxyl group into a halogen or sulphonyloxy leaving group, preferably bromide, iodide, p-toluenesulphonate, p-bromobenzenesulphonate, methane-sulphonate, and trifluoromethane-sulphonate, followed by displacement of the leaving group with a suitable nitrogen containing nucleophile such as ammonia, hydrazine or azide. Azide is the preferred nucleophile, because of the dual advantage of ease of synthesis and the possibility of further modification of the other hydroxyl groups.

(b) Direct replacement of hydroxyl group with an azide, such as treatment with triphenylphosphine and lithium azide (Boger, J. et al., Helvetica Chimica Acta, (1978) 61:2190).

(c) Opening of epoxides with a suitable nitrogen containing nucleophile such as ammonia, azide and other amines under relatively mild conditions also provides a convenient route to synthesize amino sugars.

(d) Glycosyl amines can also be easily synthesized from free sugars using ammonium-hydrogencarbonate or the appropriate protected anomerically pure glycosyl azides.

(e) Starting from a carbonyl compound (aldehyde or ketone) an amino group may be introduced via oximes or hydrazones.

The carboxylic group can be introduced by the oxidation of the primary alcohol group to a carboxylic acid by standard oxidation methodology, preferably Jones oxidation (Bowers, A. et al., J. Chem. Soc., (1953) 26:2576), platinum oxidation (Heyns, K. et al, Ber. Dtsch. Chem. Ges., (1955) 88:188) or TEMPO-mediated oxidation (de Nooy, A. E. J. et al., Carbohydrate Research, (1995) 269:89), or a single unprotected hydroxyl group can be alkylated with a carboxylic acid containing group.

Coupling of these compounds to give saccharopeptides of the general structural formula I can be accomplished by the standard methodologies of peptide chemistry using derivatives in which the amino group in one reactant and the carboxyl group in the other reactant is in suitable form to react with each other to give a peptide bond, whereas the other functional groups

could be protected. Further chain-elongation can be achieved by liberating the amino or the carboxyl group in the resulting product, and coupling it with another mono- or oligomer unit. As mentioned above, the building blocks for chain extension of the saccharo-amino acids include, but are not limited to, carbohydrates, natural amino acids, and non-natural amino acids.

Attachment of the X group to the anomeric center of the saccharide unit to form either the glycosidic or thioglycosidic or carboglycosidic bond can be achieved by the reaction of an activated saccharide derivative, e.g. glycosyl halides, thioglycosides, glycosyl imidates, or n-pentenyl glycosides with the hydroxyl or thiol of the X group. Attachment of the X group by an ether or thioether linkage can be achieved using classical methods of ether preparation.

If so desired, the saccharopeptides can be functionalized to enable the attachment of additional mono or oligosaccharide units.

An additional aspect of the present invention is the sulfation of the saccharide groups preferably via their hydroxyl or amine groups. The hydroxyl and amine groups can be either partially or completely sulfated.

After the saccharide groups have been linked by the X group(s), the saccharide groups are deprotected to yield free hydroxyls and amines. The free hydroxyls and amines are then sulfated using an appropriate sulfating agent such as but not limited to chlorosulfonic acid or complexes of sulfur trioxide with organic bases in an inert solvent such as N,N-dimethylformamide (DMF),

hexamethylphosphoric triamide, dimethyl sulfoxide (DMSO) or pyridine. Using techniques known in the art, selective sulfation of either the hydroxyl or amine groups can be obtained. In the case of sulfating amines, water can be used as a solvent. After sulfation, the sulfate groups can be modified to possess biologically acceptable cations, including but not limited to Na, K, Li, Ca, Mg, NH_4, aluminum, ethanolamine, triethanolamine, morpholine, pyridine and piperidine.

Saccharopeptides can be synthesized using solid phase synthesis methods. In general, a saccharide with a free amine is selectively protected, preferably with a Boc group by standard methodologies. The Boc-derivative is linked to the Merrifield resin as described by Merrifield. (Merrifield, R. B., Biochemistry, (1964) 3:1385; Erickson, B. W. and Merrifield, R. B., The Proteins, Neurath, H. and Hill, R. L. (eds), Vol.2, 3rd edn, Academic Press, New York, 255-527 (1979); Barany, G. and Merrifield, R. B., The Peptides, Gross, E. and Meienhofer, J. (eds), Vol.2, Academic Press, New York, 3-285 (1979).)

The Boc group is removed for further elongation, by treating the N-protected, resin linked sugar with an acid, preferably trifluoroacetic acid, to give the free amino resin linked derivative. Coupling the Boc-derivative and the free amino resin linked derivative using the previously described method yields the resin linked protected disaccharopeptide. The protecting groups are removed as described above, and the saccharopeptide is detached from the resin treating it

with hydrogen fluoride as described by Merrifield. This method can be repeated to obtain the desired length of the saccharopeptide.

Organic solvents useful for the preparation of the saccharopeptides of the instant invention include but are not limited to DMF, DMSO, 1,4-dioxane, ethyl acetate (EtOAc), hexamethylphosphoric triamide, dichloromethane, tetrahydrofuran (THF), and pyridine. TLC refers to thin layer chromatography.

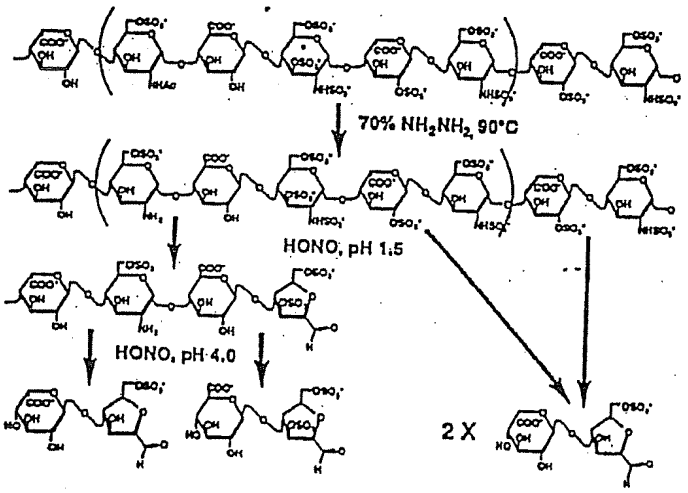
Glycoamino acids From Heparin and Related Glycosaminoglycan

Disaccharide constituents of glycosaminoglycans can be generated and isolated in a variety of ways that have been reported (B. Casu, "Structure of Heparin and Heparin Fragments" in Heparin and Related Polysaccharides, D.A. Lane, I. Bjork, and U. Lindahl, Ann. N.Y. Acad. Sci. (1989) 556:1-17). These methodologies have been applied most frequently to heparin, but are adaptable for application with other glycosaminoglycans and related polysaccharides including other glucosaminoglycans such as heparan sulfate, the K5 and K4 bacterial polysaccharides, and galactosaminoglycans such as chondroitin, chondroitin sulfates, dermatan sulfate, as well as hyaluronic acid and keratan sulfate (M. Ragazi et al J. Carbohydr. Chem. 12 (4&5) 523-535 (1993)). The following procedures for generation and isolation of heparin disaccharides illustrate this methodology and one skilled in the art would know how to adapt these methods to other glycosaminoglycan materials.

Heparin-derived Glycoaminoacids from HONO

Depolymerization of Heparin Disaccharide Generation

Nitrous acid depolymerization of heparin and related polysaccharides has been known for many years (A.B. Foster et al, J.Chem. Soc. (1963), 2279) to result in the formation of 2,5 anhydroaldose residues at the reducing end (Shively and Conrad, Biochemistry (1976) 15:3932-3942). Complete depolymerization of polysaccharide with a disaccharide repeat containing a uronic acid linked to an amino sugar, such as glycosaminoglycans, therefore yields a disaccharide derivative with the uronic acid linked to the 2,5 anhydroaldose. In the case of heparin this terminal anhydroaldose derived from glucosamine is anhydromannose. For the galactosaminoglycans such as chondroitin and dermatan, the galactosamine derived reducing end is anhydrotalose. Conrad et al (Anal. Biochemistry, (1989) 176, 96-104.) as well as others have reported efficient methodology for the complete depolymerization of heparin using the nitrous acid procedure. This is outlined in the schematic below.



EXAMPLES

Example 1

Synthesis of saccharo amino acids - 1-amino-1-deoxy uronic acid derivatives

Methyl (2,3,4-tri-O-acetyl-β-D-glucopyranosyl azide)uronate (1)

Methyl {2,3,4-tri-O-acetyl-β-D-glucopyranosyl bromide}uronate (8 g) was dissolved in DMF (100 ml) and a 1:1 mixture of sodium azide (3.25 g): lithium azide (2.25 g) was added into the solution. The mixture was stirred overnight at room temperature and was diluted with chloroform (500 ml) and water. The organic layer was washed with water and evaporated. The residue was crystallized from ethanol to yield **1** (6.67 g, 92 %). $[\alpha]_D$ -33.4^0 (*c* 1.00, chloroform); ^{1}H-NMR data ($CDCl_3$): δ 2.03, 2.04, and 2.08 (3s, 3H, 3 $COCH_3$), 3.78 (s, 3H, $COOCH_3$), 4.13 (d, 1H), 4.73 (d, 1H, H-1 $J_{1,2}$ = 8.7 Hz), 4.97 (t, 1H), 5.25 (m, 2H). ^{13}C-NMR data ($CDCl_3$): δ 20.5, 20.6 (3C, $COCH_3$), 53.1 ($COOCH_3$), 69.0, 70.4, 71.8, 74.2 (4C, C-2,3,4,5), 88.1 (C-1), 166.5 ($COOCH_3$), 169.1, 169.3, 170.0 (3C, $COCH_3$).

Methyl (2,3,4-tri-O-acetyl- -D-glucopyranosyl amine)uronate (2)

A solution of **1** (0.48 g) in EtOAc (10 ml) was hydrogenated in the presence of 10% palladium on carbon (Pd-C, 0.1 g) at room temperature and atmospheric pressure for one hour. The catalyst was filtered off and the

filtrate was concentrated to a syrup 2 (0.44 g, 100 %). ^{1}H-NMR data ($CDCl_3$): δ 2.02, 2.06 (2s, 9H, 3 $COCH_3$), 3.74 (s, 3H, $COOCH_3$), 4.04 (d, 1H), 4.25 (d, 1H), 4.87 (t, 1H), 5.17 (m, 1H), 5.31 (t, 1H). ^{13}C-NMR data ($CDCl_3$): δ 20.5, 20.6, 20.8 (3C, $COCH_3$), 52.9 ($COOCH_3$), 61.0, 71.7, 72.4, 73.5 (4C, C-2,3,4,5), 85.3 (C-1).

2,3,4-Tri-O-acetyl-1-azido-1-deoxy-β-D-glucopyranuronic acid (6)

(a) To a solution of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl azide (3, 5.6 g) in methanol (50 ml) 1 M methanolic sodium methoxide (0.5 ml) was added and the solution was stirred overnight at 0°C, then it was neutralized with AG 50W-X8 (H^+) ion-exchange resin. The resin was filtered off, the filtrate was evaporated and dried in vacuo to give the known β-D-glucopyranosyl azide (4).

Composition 4 (3.05 g) was dissolved in dry pyridine (50 ml), chlorotriphenyl-methane (5.01 g) was added and the reaction mixture was stirred at 70°C for three hours. It was cooled to 0°C, acetic anhydride (6.36 ml) was added dropwise and the mixture was stirred overnight at room temperature. It was poured into ice-water and was diluted with chloroform (300 ml). The organic layer was washed subsequently with water, 2 M hydrochloric acid, water, was dried and evaporated to leave 2,3,4-tri-O-acetyl-6-O-trityl-β-D-glucopyranosyl azide (5, 7.67 g, 90 %).

Jones oxidation of composition 5 (7.67 g) in acetone (70 ml) with a solution of chromium trioxide (8.02 g) in 3.5 M sulfuric acid (10 ml) afforded the acid 6 which was purified by column chromatography to give 2.86 g (62%)

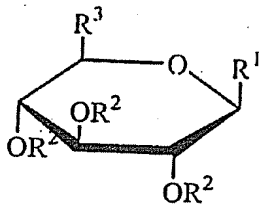
final product. $[\alpha]_D$ -12.8° (*c* 1.00, methanol), ^{1}H-NMR data (CD_3OD): δ 1.98, 2.00, 2.05 (3s, 9H, $COCH_3$), 4.25 (d, 1H), 4.95 (m, 2H,), 5.19 (t, 1H), 5.34 (t, 1H). ^{13}C-NMR data (CD_3OD): δ 20.5, 20.6 (3C, $COCH_3$), 70.7, 72.0, 73.7, 75.4 (C-2,3,4,5), 88.8 (C-1), 170.9, 171.1, 171.4 (3C, $COCH_3$).

(b) Selective deesterification of (1) (371 mg) with lithium iodide (670 mg) in pyridine (5 ml) gave the free carboxylic acid (6) (165 mg, 47 %).

1-Azido-1-deoxy 2,3,4-tri-O-benzoyl-β-D-glucuronic acid (7)

To a solution of composition 4 (3.51 g) in pyridine (50 mL) at 70 °C, then it was cooled to 0°C and benzoyl chloride (10.37 mL) was added dropwise to the solution. The reaction mixture was stirred overnight at room temperature, then was poured into ice-water and diluted with chloroform (500 mL). The organic layer was separated and washed with 2M hydrochloric acid, water, dried and evaporated. The crude product, 2,3,4-tri-O-benzoyl-6-O-trityl-β-D-glucopyranosyl azide (11.40 g) was directly oxidized, using the Jones method, at 0 °C in aceton (250 mL) with a solution of chromium trioxide (8.02 g) in 3.5 M sulfuric acid. The resulting product was purified by column chromatography (toluene-2-propanol, 4:1) to give 1-azido-1-deoxy 2,3,4-tri-O-benzoyl-β-D-glucuronic acid (7.42 g, 89%), ^{1}H-NMR ($CDCl_3$): δ 4.25 (bd, 1H), 5.01 (d, 1H, H-1 $J_{1,2}$=9.0 Hz), 5.50 (dt, 1H), 5.80 (m, 2H), 7.12-8.31 (m, 15H); ^{13}C-NMR ($CDCl_3$): δ 70.14, 71.40, 72.88, 75.65 (C-2,3,4,5), 88.77 (C-1), 128.18, 128.42, 128.88, 129.07, 129.75, 129.97, 130.18, 133.04, 133.31 (*COPh*), 164.92, 165.68 (3C, *COPh*), 166.11 (*COOH*).

Structures of 1-amino-1-deoxy uronic acid derivatives



1	$R_1 = N_3$	$R_2 = Ac$	$R_3 = CO_2Me$
2	$R_1 = NH_2$	$R_2 = Ac$	$R_3 = CO_2Me$
3	$R_1 = N_3$	$R_2 = Ac$	$R_3 = CH_2OAc$
4	$R_1 = N_3$	$R_2 = H$	$R_3 = CH_2OH$
5	$R_1 = N_3$	$R_2 = Ac$	$R_3 = CH_2OTr$
6	$R_1 = N_3$	$R_2 = Ac$	$R_3 = CO_2H$
7	$R_1 = N_3$	$R_2 = Bz$	$R_3 = CO_2H$

Example 2

Synthesis of saccharo amino acids - 2-amino-2-deoxy uronic acid derivatives

Methyl 3,4-di-O-acetyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranosyluronic acid (10)

To a solution of methyl 2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside (8, 6.36 g) in dry pyridine (30 ml) triphenylmethyl chloride (8.12 g) was added. The mixture was stirred at 70°C for three hours. After cooling to room temperature acetic anhydride (5.49 ml) was added and the mixture was stirred overnight at room temperature. It was poured into ice-water and worked up as described or

5. Column chromatography (toluene-ethyl acetate, 85:15) afforded methyl 3,4-di-O-acetyl-2-benzyloxycarbonylamino-2-deoxy-6-O-triphenylmethyl-α-D-glucopyranoside (9, 11.65 g, 96 %).

Jones oxidation of composition 9 (11.65 g) in acetone (100 ml) with a solution of chromium trioxide (10.7 g) in 3.5 M sulfuric acid (15 ml) after purification by column chromatography gave the acid 10, (5.36 g, 71 %). $[\alpha]_D$ +66.2 (c 1.03 methanol); ^{1}H-NMR data (CD_3OD): δ 1.81, 1.99 (2s, 6H, $COCH_3$), 3.43 (s, 3H, OCH_3), 4.10 (dd, 1H, H-2), 4.14 (d, 1H, H-5), 4.96 (d, 1H, H-1, $J_{1,2}$ = 3.3 Hz), 5.01 (d, 1H, OCH_2), 5.16 (d, 1H, OCH_2), 5.18 (t, 1H, H-3), 5.25 (t, 1H, H-4). ^{13}C-NMR data (CD_3OD): δ 20.6, 20.9 (2C, $COCH_3$), 54.6 (OCH_3), 56.5 (C-2), 67.6 (OCH_2Ph), 71.2, 71.5, 72.6 (C-3,4,5), 100.3 (C-1), 128.9, 129.0, 129.4 (aromatic carbons), 138.3 (quaternary aromatic carbon), 158.3 ($OCOCH_2Ph$), 171.6, 171.9 (2C, $COCH_3$), 175.7 (COOH).

Methyl (methyl 3,4-di-O-acetyl-2-amino-2-deoxy-α-D-glucopyranoside)uronate (12)

To a solution of 10 (1.8 g) in dry methanol AG 50W-X8 (H^+) ion-exchange resin was added and mixture was stirred overnight. The resin was filtered off and the filtrate was evaporated. The residue was purified by column chromatography (toluene-ethyl acetate, 3:2) to give methyl (methyl 3,4-di-O-acetyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside)uronate (11,1.68 g, 93 %). $[\alpha]_D$ +92.5 (c 1.04 chloroform); ^{1}H-NMR data ($CDCl_3$): δ 1.87, 1.99 (2s, 6H, $COCH_3$), 3.40 (s, 3H, OCH_3), 3.72 (s, 3H, $COOCH_3$), 4.09 (m, 1H, H-2), 4.25 (d, 1H, H-5), 4.84 (d, 1H, H-1, $J_{1,2}$ = 3.3 Hz), 5.01 (d, 1H, OCH_2), 5.14 (d, 1H, OCH_2),

H-1, $J_{1,2}$ = 3.3 Hz), 5.01 (d, 1H, OCH_2), 5.14 (d, 1H, OCH_2), 5.16 (t, 1H, H-4), 5.25 (t, 1H, H-3), 5.30 (d, 1H, NH). ^{13}C-NMR data ($CDCl_3$): δ 20.9 (s, 2C, $COCH_3$), 53.2 ($COOCH_3$), 53.9 (C-2), 56.4 (OCH_3), 67.3 (OCH_2), 69.0 (C-5), 69.9 (C-4), 70.9 (C-3), 99.3 (C-1), 128.5, 128.6, 129.0 (aromatic carbons), 136.9 (quaternary aromatic carbon), 156.2 ($OCOCH_2Ph$), 168.6 ($COOCH_3$), 169.8, 171.1 ($COCH_3$).

A solution of 11 (219 mg) in EtOAc (5 ml) was hydrogenated in the presence of 10 % Pd-C (0.1 g) at room temperature and at atmospheric pressure for one hour. The catalyst was filtered off and the filtrate was evaporated to give 12 as a syrup (157 mg, 100%). $[\alpha]_D$ +148.3 (c 1.26 chloroform); ^{1}H-NMR data ($CDCl_3$): δ 1.53 (s, 2H, NH_2), 2.02, 2.09 (2s, 6H, $COCH_3$), 2.97 (dd, 1H, H-2), 3.46 (s, 3H, OCH_3), 3.75 (s, 3H, $COOCH_3$), 4.29 (d, 1H, H-5), 4.84 (d, 1H, H-1, $J_{1,2}$ = 3.4 Hz), 5.06 (t, 1H, H-4), 5.17 (t, 1H, H-3). ^{13}C-NMR data ($CDCl_3$): δ 20.6, 20.9 (2s, $COCH_3$), 52.8 ($COOCH_3$), 54.3 (C-2), 56.0 (OCH_3), 68.7, 69.9, 73.6 (3C, C-5,4,3), 101.0 (C-1), 168.6 ($COOCH_3$), 169.7, 170.7 (2C, $COCH_3$).

Methyl 3,4-di-O-benzoyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside uronic acid (14)

Methyl 2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside (4.91 g) was dissolved in pyridine (20 mL), chlorotriphenylmethane (6.30 g) was added to the solution and the reaction mixture was stirred overnight at 70 °C. The reaction mixture was cooled to 0 °C, benzoyl chloride (5.22 mL) was added dropwise and the mixture was stirred overnight at room temperature. It was poured into ice-water and extracted with chloroform (2 x 200 mL). The organic layer was separated and was washed with 2M

hydrochloric acid, water, dried, the solvent was evaporated and the crude product was purified by column chromatography (toluene-ethyl acetate, 95:5→9:1) to give methyl 3,4-di-O-benzoyl-2-benzyloxycarbonylamino-2-deoxy-6-O-trityl-α-D-glucopyranoside (13), (0.99 g 94%); ^{1}H-NMR ($CDCl_3$): δ 3.43 (s, OCH_3), 3.94 (t, 1H), 4.12 (ddd, 1H), 4.14 (dd, 1H), 4.28 (dd, 1H), 4.38 (dd, 1H, H-2), 4.91 (d, 1H, $PhCH_2$), 4.93 (d, 1H, H-1 $J_{1,2}$= 3.5 Hz), 5.04 (d, 1H, $PhCH_2$), 5.45 (d, 1H, NH, $J_{2,NH}$=9.8 Hz), 5.80 (t, 1H), 7.15-7.50 (m, 30H, *Ph*); ^{13}C-NMR ($CDCl_3$): δ 54.05, 55.42 (OCH_3, C-2), 62.54 (C-6), 67.12 ($PhCH_2$), 69.61, 70.32, 71.82 (C-3,4,5), 86.64 ($C(Ph)_3$), 98.73 (C-1), 127.68-129.84 (*Ph*), 136.11 (C_qPhCH_2), 143.65 ($C_qC(Ph)_3$), 155.94 ($PhCH_2OCO$), 166.08, 166.57 (*CO*Ph).

Jones oxidation of composition **13** (9.99 g) with a solution of chromium trioxide (8.98 g) in 3.5M sulfuric acid (10 mL) in acetone gave the crude title product which was purified by column chromatography (toluene-2-propanol 9:1→3:2) to give the free uronic acid **14** (4.80 g, 68%); ^{1}H-NMR (CD_3OD): δ 3.48 (s, OCH_3), 4.41 (d, 1H, H-5), 4.54 (dd, 1H, H-2), 4.88 (d, 1H, OCH_2), 4.96 (d, 1H, H-1 $J_{1,2}$ =3.8 Hz), 5.05 (d, 1H, OCH_2), 5.66 (dd, 1H), 5.85 (t, 1H), 7.25-7.58 (m, 11H, *Ph*), 7.85, 7.95 (2d, 4H, *Ph*); ^{13}C-NMR (CD_3OD): δ 53.41, 55.89 (OCH_3, C-2), 67.50 (OCH_2), 69.48, 72.18 (2C), (C-3,4,5), 101.84 (C-1), 128.63-134.41 (17C, *Ph*), 138.06 (C_qPhCH_2), 158.55 ($PhCH_2OCO$), 166.90, 166.96 (2C, *CO*Ph), 175.68 (*COOH*), FAB-MS (mNBA+NaOAc): $[M-H]^-$ 548.4, $[M+Na]^+$ 572.2.

Methyl 3,4-di-O-acetyl-2-(9-fluorenylmethoxy-carbonyl)amino-2-deoxy-α-D-gluco-pyranoside uronic acid (17)

D-Glucosamine hydrochloride (13.57 g) and sodium hydrogencarbonate (10.59 g) were dissolved in water (45 mL), cooled to 0 °C, and a solution of Fmoc-chloride (19.70 g) in 1,4-dioxane (50 mL) was added dropwise. The reaction mixture was stirred overnight at room temperature, the precipitated white solid was collected by filtration and recrystallized from 80% methanol to give the Fmoc protected glucoseamine (21.68 g, 90%).

To form the methyl glycoside 7.00 g of the above product was refluxed overnight in methanol containig 1% HCl, then the solution was cooled to room temperature and neutralized with sodium bicarbonate. The solid was filtered off, the solvent was evaporated and the crude product was recrystallized from 95% ethanol to give methyl 2-(9-fluorenylmetoxycarbonyl)amino-2-deoxy-α-D-glucopyranoside (15) (6.54 g, 90%), $[\alpha]_D$ +58.8 (*c* 1.17, chloroform).

Composition 15 was converted into the acetylated 6-O-trityl derivative as described earlier, reacting the free sugar with chlorotriphenylmethane (4.18 g) in pyridine (25 mL) to give 6.69 g (90%) methyl 3,4-di-O-acetyl-2-(9-fluorenylmetoxycarbonyl)amino-2-deoxy-6-trityl-α-D-glucopyranoside (16), ^{13}C-NMR ($CDCl_3$): δ 47.20 (Fmoc*CH*), 54.03, 55.29 (O*CH*$_3$, C-2), 62.39 (C-6), 67.18 (Fmoc*CH*$_2$), 68.97, 69.24, 71.71 (C-3,4,5), 86.70 (*C*(Ph)$_3$), 98.42 (C-1), 125.16-129.92 (8C, *Fmoc*CH$_2$), 141.39 (4C$_{ii'}$

FmocCH_2), 143.79, 143.82, 143.85, 143.91 ($5C_qC(Ph)_3$, FmocCH$_2$), 155.97 (FmocCH$_2$O*CO*), 169.10, 171.24 (*CO*CH_3).

Composition 16 (6.69 g) was converted to the uronic acid by Jones-oxidation (4.50 g of chromium trioxide in 8 mL of 3.5M sulfuric acid) to give the title product (17), ^{1}H-NMR (CD_3OD): δ 1.82, 1.99 (2s, CO*CH*$_3$), 3.45 (s, O*CH*$_3$), 4.15 (m, 4H, H-5, Fmoc*CH*, Fmoc*CH*$_2$), 4.39 (dd, 1H, H-2), 5.01 (d, 1H, H-1 $J_{1,2}$ =3.3 Hz), 5.29 (2t, 2H, H-3,4), 7.19-7.37 (m, 4H, *Ph*), 7.52, 7.64 (2d, 4H, *Ph*); ^{13}C-NMR (CD_3OD): δ 20.85, 20.98 (2C, *C*OCH$_3$), 48.14 (Fmoc*CH*), 54.48, 56.64 (O*CH*$_3$, C-2), 68.18 (Fmoc*CH*$_2$), 71.12, 71.49, 72.35 (C-3,4,5), 100.19 (C-1), 120.89, 126.15, 126.23, 128.12, 128.73 (8C, *Fmoc*CH$_2$), 142.43, 145.03, 145.21 (4C$_q$, *Fmoc* CH$_2$), 158.25 (FmocCH$_2$O*CO*), 171.58, 171.97 (2C, *CO*CH$_3$), 175.98 (*CO*OH).

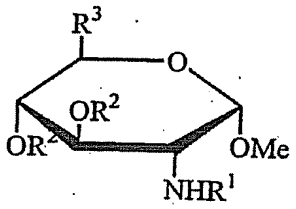
Methyl 3,4-di-O-benzoyl-2-(9-fluorenylmethoxycarbonyl) amino-2-deoxy-α-D-glucuronic acid (19)

Composition 15 (4.15 g) was reacted in pyridine (25 mL) with chlorotriphenylmethane (4.15 g) followed by benzoyl chloride (3.46 mL) as described earlier to give the benzoylated trityl derivative (18) (7.45 g, 86%), ^{1}H-NMR ($CDCl_3$): δ 3.41 (s, OCH_3), 3.91 (t, 1H, Fmoc*CH*), 4.07 (dd, 1H), 4.12 (m, 1H), 4.30 (dd, 1H), 4.40 (dd,1H, H-2), 4.90 (d, 1H, $J_{1,2}$= 3.3 Hz), 5.44 (d, 1H, NH, $J_{2,NH}$=9.9 Hz), 5.63, 5.71 (2t, H-3,4), 7.12-7.42 (m, 34H, *Ph*), 7.61, 7.66, 7.79 (3d, 4H); ^{13}C-NMR ($CDCl_3$): δ 46.92 (Fmoc*CH*), 54.43, 55.72 (O*CH*$_3$, C-2), 62.52 (C-6), 67.02 (Fmoc*CH*$_2$), 69.42, 69.64, 72.21 (C-3,4,5), 86.64 (*C*(Ph)$_3$), 98.54 (C-1), 119.78, 124.90, 125.05, 125.26, 126.85-129.91 (33C, *Ph*,

Fmoc), 137.67, 141.04, 141.13, 143.47, 143.64, 143.88 (9C_q, *Fmoc*CH_2, C(*Ph*)$_3$, *Ph*), 155.93 (Fmoc *C*=*O*), 164.84, 166.82 (Ph*CO*).

Composition **18** (6.49 g) was oxidized using the previously used method to give 2.96 g (62%) of the title compound (**19**), ^{13}C-NMR (CD_3OD): δ 46.79 (Fmoc*CH*), 53.57, 56.09 (O*CH*$_3$, C-2), 67.08 (Fmoc*CH*$_2$), 70.29, 71.64 (C-3,4,5), 99.06 (C-1), 119.72, 124.94-132.34 (18C, *Fmoc*, CO*Ph*), 140.97, 141.04 (C_q, CO*Ph*) 143.65, 143.90 (4C_q, *Fmoc*), 155.87 (Fmoc *CO*), 164.43, 166.61 (2C, *CO*Ph), 175.54 (*C*OOH).

Structures of 2-amino-2-deoxy uronic acid derivatives



8	R_1 = Z	R_2 = OH	R_3 = CH_2OH
9	R_1 = Z	R_2 = Ac	R_3 = CH_2OTr
10	R_1 = Z	R_2 = Ac	R_3 = CO_2H
11	R_1 = Z	R_2 = Ac	R_3 = CO_2Me
12	R_1 = H	R_2 = Ac	R_3 = CO_2Me
13	R_1= Z	R_2 = Bz	R_3 = CH_2OTr
14	R_1= Z	R_2 = Bz	R_3 = CO_2H
15	R_1 = Fmoc	R_2 = OH	R_3 = CH_2OH
16	R_1 = Fmoc	R_2 = Ac	R_3 = CH_2OTr
17	R_1 = Fmoc	R_2 = Ac	R_3 = CO_2H
18	R_1 = Fmoc	R_2 = Bz	R_1 = CH_2OTr
19	R_1 = Fmoc	R_2 = Bz	R_3 = CO_2H

Example 3

Synthesis of saccharo amino acids - 3-amino-3-deoxy uronic acid derivatives

Methyl 2,4-di-O-benzoyl-3-azido-3-deoxy-β-D-glucuronic acid (27)

1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (20.8 g) was oxidized in a mixture of ethyl acetate (100 mL) and dimethyl sulfoxide (60 mL) to 1,2:5,6-di-O-isopropylidene-α-D-*ribo*-hexofuranose-3-ulose with DCC (41.30 g) in the presence of anhydrous phosphoric acid (4.00 g). The precipitated DCU was filtered off, the solution was washed with sat. sodium bicarbonate, brine and the solvent was evaporated.

The same compound was also synthesized by oxidation of the starting material (50.0 g) with acetic anhydride and dimethyl sulfoxide.

The crude product was reduced by stirring with sodium borohydride (3.00 g) in 95% ethanol (300 mL) for two hours at room temperature. The solvent was evaporated, the residue was partitioned between chloroform and water. The organic layer was separated, was washed with water, dried and evaporated to provide 1,2:5,6-di-O-isopropylidene-α-D-allofuranose (46.52 g 93%). This was converted into the 3-O-tosyl derivative by reaction in pyridine (120 mL) with *p*-toluenesulphonyl chloride (40.90 g) overnight at room temperature. The reaction mixture was poured into ice-water, the solid was collected and recrystallized from ethanol to give provide 1,2:5,6-di-O-isopropylidene-3-O-tosyl-α-D-allofuranose (20) (61.17 g,

82%), ^{1}H-NMR ($CDCl_3$): δ 1.23, 1.24, 1.26, 1.50 (s, 2 $C(CH_3)_2$), 2.42 s, (CH_3 tosyl), 3.76 (dd, 1H), 3.91 (dd, 1H), 4.61 (m, 2H, dd, H-1 $J_{1,2}$=3.3 Hz), 5.74 (d, 1H), 7.35 (2d, 4H, *Ar tosyl*); ^{13}C-NMR ($CDCl_3$): δ 21.58 (CH_3 tosyl), 25.01, 26.05, 26.53, 26.61 ($C(CH_3)_2$), 65.15 (C-6), 74.70, 76.81, 77.00, 77.90 (C-2,3,4,5), 103.83 (C-1), 109.72, 113.43 ($C(CH_3)_2$), 128.27, 129.68 (Ar *tosyl*), 145.19 (C_q Ar *tosyl*).

Composition **20** (28.00 g) was reacted with sodium azide (54.00 g) in N,N-dimethylformamide (400 mL) at 120 °C until the staring material was fully converted. The solvent was evaporated, the residue was taken up in chloroform (500 mL) and extracted with water, then the solvent was evaporated. The crude product was purified by column chromatography (toluene-ethyl acetate, 85:15) to give 3-azido-3-deoxy-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (**21**) (15.13 g, 78 %), ^{1}H-NMR ($CDCl_3$): δ 1.32, 1.36, 1.44, 1.51 (s, 2 $C(CH_3)_2$), 3.98 (dd, 1H), 4.12 (m, 3H), 4.24 (ddd, 1H, H-5), 4.62 (d, 1H, H-1 $J_{1,2}$=3.5 Hz), 5.86 (d, 1H); ^{13}C-NMR ($CDCl_3$): δ 25.16, 26.20, 26.56, 26.87 ($C(CH_3)_2$), 67.65 (C-6), 66.36, 73.05, 80.49, 83.42 (C-2,3,4,5), 105.05 (C-1), 109.58, 112.31 ($C(CH_3)_2$).

The isopropylidene protecting groups were removed by refluxing composition **21** (7.12 g) in 75% acetic acid to give the unprotected 3-azido-3-deoxy-D-glucose (**22**) (5.00 g, 97%), ^{1}H-NMR (D_2O): δ 4.67 (d, 1H, H-1b $J_{1,2}$=7.8 Hz), 5.20 (d, 1H, H-1a $J_{1,2}$ =3.6 Hz); ^{13}C-NMR (D_2O): δ 60.59, 60.77 (2C, C-6), 66.12, 68.71, 68.83, 70.57, 71.46, 73.03, 75.74 (8C, C-2,3,4,5), 91.71, 96.15 (C-1), which was acetylated in pyridine (15 mL) with acetic anhydride (18.00 mL) to give the 1,2,4,6-tetra-O-acetyl-3-azido-3-

deoxy-D-glucose (**23**) (8.32 g, 93%), ^{1}H-NMR ($CDCl_3$): δ 5.57 (d, 1H, H-1b $J_{1,2}$=8.3 Hz), 6.31 (d, 1H, H-1a $J_{1,2}$=3.6 Hz); ^{13}C-NMR ($CDCl_3$): δ 20.46, 20.57, 20.61, 20.70, 20.79, 20.83 (8C, $COCH_3$), 61.49 (2C, C-6), 60.81, 67.75, 67.81, 69.96; 70.00, 70.08, 73.54 (8C, C-2,3,4,5), 88.69, 91.88 (C-1), 168.60, 168.95, 169.04, 169.11, 169.15, 169.40, 170.64 ($COCH_3$).

Composition **23** (6.48 g) was converted into the glycosyl bromide, by reacting it in dichloromethane (25 mL) with hydrobromic acid in acetic acid (8.31 mL) at 0 °C. The reaction was worked up as usual, and the crude bromide was reacted with methanol (7.20 mL) in the presence of mercury(II) oxide (1.00 g) and mercury(II) bromide (0.07 g). After full conversion of the bromide, the reaction mixture was diluted with chloroform (300 mL), filtered through a pad of Celite, the filtrate was washed with 10% aq. potassium iodide, water, sat. aq. sodium bicarbonate, water, the solution was dried and the solvent was evaporated. The residue was separated on a silica gel column (toluene-ethyl acetate, 9:1→85:15) to give methyl 2,4,6-tri-O-acetyl-3-azido-3-deoxy-β-D-glucopyranoside (**24**) (1.92 g, 32%), ^{1}H-NMR ($CDCl_3$): δ 1.99, 2.03, 2.05 (3s, $COCH_3$), 3.40 (s, OCH_3), 3.58 (t, 1H, H-3 $J_{3,4}$=10.0 Hz), 3.61 (ddd, 1H, H-5), 4.02 (dd, 1H, H-6b $J_{5,6b}$=2.3 Hz), 4.16 (dd, 1H, H-6a $J_{5,6a}$=4.8 Hz), 4.32 (d, 1H, H-1 $J_{1,2}$=7.8 Hz), 4.80 (dd, 1H, H-2 $J_{2,3}$=8.3 Hz), 4.89 (t, 1H, H-4 $J_{4,5}$=9.9 Hz); ^{13}C-NMR ($CDCl_3$): δ 20.61, 20.68, 20.71 (8C, $COCH_3$), 56.90 (OCH_3), 62.01 (C-6), 64.28, 68.54, 71.07, 72.58 (C-2,3,4,5), 101.77 (C-1), 169.15, 169.27, 170.61 ($COCH_3$).

Composition 24 (1.86 g) was deprotected in methanol (10 mL) with sodium methoxide at pH=9. The solution was neutralized and evaporated to provide 1.18 g (98%) methyl 3-azido-3-deoxy-β-D-glucopyranoside (25), ^{1}H-NMR (D_2O): δ 3.26 (dd, 1H, H-2), 3.47 (2t, 2H, H-3,4), 3.50 (ddd, 1H, H-5), 3.56 (s, 3H, OCH_3), 3.70 (dd, 1H, H-6a), 3.89 (dd, 1H, H-6b), 4.42 (d, 1H, H-1 $J_{1,2}$=8.0 Hz); ^{13}C-NMR ($CDCl_3$): δ 57.42 (OCH_3), 60.75 (C-6), 68.83, 68.92, 71.99, 76.71 (C-2,3,4,5), 103.41 (C-1). This was tritylated (chlorotriphenylmethane 3.00 g) in pyridine (10 mL), then benzoylated (benzoyl chloride 2.25 mL) to provide methyl 3-azido-2,4-di-O-benzoyl-3-deoxy-6-O-trityl-β-D-glucopyranoside (26) (3.31 g 92%), ^{1}H-NMR ($CDCl_3$): δ 3.23 (dd, 1H, H-6a, $J_{5,6a}$=4.8 Hz), 3.31 (dd, 1H, H-6b $J_{5,6b}$=2.3 Hz), 3.51 (s, 3H, OCH_3), 3.75 (ddd, 1H, H-5), 3.92 (t, 1H, H-3 $J_{3,4}$=10.0 Hz), 4.62 (d, 1H, H-1 $J_{1,2}$=7.8 Hz), 5.31 (dd, 1H, H-2 $J_{2,3}$ =10.0 Hz), 5.41 (t, 1H, H-4 $J_{4,5}$ =8.9 Hz), 7.51-7.59 (m, 21H, *Ph*), 7.82, 8.04 (2d, 4H, *Ph*); ^{13}C-NMR ($CDCl_3$): δ 56.30 (OCH_3), 62.12 (C-6), 64.62 (C-3), 69.24 (C-4), 71.61 (C-2), 74.24 (C-5), 86.42 ($C(Ph)_3$), 101.61 (C-1), 126-65, 126.96, 127:44, 127.65, 128.07, 128.19, 128.30, 129.49, 129.66, 133.15 (*Ph*), 143.26 (C_q $C(Ph)_3$), 146.60 (C_q *COPh*), 164.38, 164.69 (*CO*Ph).

Composition 26 (3.28 g) was oxidized in aceton (100 mL) with a 3.5 M sulfuric acid solution of chromium trioxide (1.47 g) and the mixture was worked up as previously described to obtain the title compound (27), (1.51 g, 70%), ^{1}H-NMR (CD_3OD): δ 3.45 (s, 3H, OCH_3), 4.25 (d, 1H, H-5 $J_{4,5}$=10.0 Hz), 4.26 (t, 1H, H-3 $J_{3,4}$=9.8 Hz), 4.84 (d, 1H, H-1 $J_{1,2}$=7.9 Hz), 5.22 (dd, 1H, H-2 $J_{2,3}$=10.1 Hz), 4.56 (t, 1H, H-4), 7.48 (m, 4H, *Ph*), 7.61 (m, 2H,

Ph), 8.11 (d, 4H, *Ph*); ^{13}C-NMR (CD_3OD): δ 57.70 (OCH_3), 65.64 (C-3), 72.25 (C-2), 73.00 (C-4), 75.97 (C-5), 102.96 (C-1), 128.55, 128.67, 130.48, 130.55, 130.74, 130.91 (*Ph*), 134.63, 134.70 (C_q *COPh*), 166.45, 166.67 (*CO*Ph), 173.80 (*COOH*).

Methyl 2,4-di-O-acetyl-3-(9-fluorenylmethoxycarbonyl) amino-3-deoxy-β-D-glucuronic acid (32)

Composition 21 (2.85 g) was hydrogenated in 10% aq. methanol (100 mL) in the presence of 10% palladium on activated carbon at atmospheric pressure. The catalyst was filtered off and the solvent was evaporated. The crude product was dissolved in a mixture of water-dioxane 1:1 (30 mL), sodium bicarbonate (1.68 g) was added into the solution and the mixture was stirred until the solid was dissolved, then it was cooled to 0°C and a solution of FmocCl (2.98 g) in 1,4-dioxane (25 mL) was added dropwise to the solution and the mixture was stirred overnight. The reaction mixture was diluted with chloroform containig 20% of tetrahydrofuran (250 mL) and the organic phase was separated and washed with water. Column chromatography (toluene-ethyl acetate, 6:5) gave 1,2:5,6-di-O-isopropylidene-3-(9-fluorenylmethoxycarbonyl)amino-α-D-glucofuranose (4.18 g). This was treated with 75% acetic acid to hydrolize the isopropylidene groups and the resulting product was acetylated in pyridine (25 mL) with acetic anhydride. The mixture was worked up as described previously to provide 2,4,6-tri-O-acetyl-3-(9-fluorenylmethoxycarbonyl)amino-3-deoxy-D-glucopyranose (4.75 g, 96 %). This compound was converted the glycosyl bromide derivative by reaction with titanium terabromide

in a mixture of dichloromethane ethyl acetate 2:1 (225 mL), which was transformed into the methyl glycoside by reacting the bromide with methanol (5.2 mL) in dichloromethane (15 mL) in the presence of silver trifluoromethanesulfonate (2.57 g). The crude product was purified by column chromatography (toluene-ethyl acetate, 85:15) to give the methyl 2,4,6-tri-O-acetyl-3-(9-fluorenylmethoxycarbonyl)amino-2-deoxy-β-D-glucopyranoside (**28**) (4.38 g, 97%), ^{1}H-NMR ($CDCl_3$): δ 1.98, 2.02, 2.08 (3s, 9H, $COCH_3$), 3.51 (s, 3H, OCH_3), 3.77 (ddd, 1H, H-5), 4.09 (dd, 1H, H-3), 4.15 (dd,t, 2H, H-6a, Fmoc*CH*), 4.28 (2dd, 2H, FmocCH_2), 4.32 (dd, 1H, H-6b), 4.48 (d, 1H, H-1 $J_{1,2}$=7.7 Hz), 4.89 (dd, 1H, H-2), 5.00 (t, 1H, H-4), 5.39 (d, 1H, NH), 7.29, 7.37 (2t, 4H, *Ph*), 7.53 (d, 2H, *Ph*), 7.73 (d, 2H, *Ph*); ^{13}C-NMR ($CDCl_3$): δ 20.66, 20.70, 20.78 ($COCH_3$), 46.87 (Fmoc*CH*), 55.69 (C-3), 56.94 (OCH_3), 62.22 (C-6), 67.42 (FmocCH_2), 68.71 (C-4), 71.55 (C-2), 72.77 (C-5), 102.10 (C-1), 119.97, 125.10, 127.13, 127.73 (*Ph*), 141.20, 143.74 (C_q, Ph), 156.40 (Fmoc*OCO*), 170.19, 170.27, 170.67 ($COCH_3$).

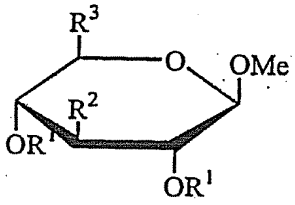
Deacetylation of composition **28** (4.33 g) with sodium methoxide in methanol provided two products, methyl 2-O-acetyl-3-(9-fluorenylmethoxycarbonyl)amino-3-deoxy-β-D-glucopyranoside (**29**) (0.76 g, 20%), ^{1}H-NMR (CD_3OD): δ 1.68 ($COCH_3$), 3.09 (m, 1H, H-4), 3.16 (ddd, 1H, H-5), 3.28 (s, 3H, OCH_3), 3.46 (dd, 1H, H-6b), 3.51 (dd, 1H, H-3), 3.68 (dd, 1H, H-6a), 4.00 (t, dd, 2H, Fmoc*CH* and CH_2), 4.52 (dd, 1H, FmocCH_2), 4.21 (d, 1H, H-1 $J_{1,2}$=8.0 Hz), 4.53 (dd, 1H, H-2), 7.06, 7.14 (2t, 4H, *Ph*), 7.44 (dd, 2H, *Ph*), 7.58 (d, 2H, *Ph*); ^{13}C-NMR ($CDCl_3$): δ 20.80 ($COCH_3$), 47.02 (Fmoc*CH*), 57.02 (OCH_3), 57.87 (C-3), 61.53 (C-6), 67.26 (Fmoc CH_2),

69.19, 71.80, 76.80, (C-2,4,5), 102.24 (C-1), 119.97, 125.14, 127.12, 127.73 (*Ph*), 141.27, 143.82 (C_q, *Ph*), 157.73 (Fmoc *CO*), 170.85 (*COCH*$_3$); and methyl 3-(9-fluorenylmethoxycarbonyl)-amino-3-deoxy-β-D-glucopyranoside (**30**) (2.60 g, 78%), ^{1}H-NMR ($CDCl_3$): δ 3.32 (O*CH*$_3$), 3.35 (m, 2H), 3.38 (m, 2H), 3.82 (m, 3H, incl. dd, ddd, H-6, H-3 respectively), 4.22 (t, 1H), 4.27 (d, 1H, H-1 $J_{1,2}$=7.7 Hz), 4.42 (2d, 2H), 7.31 (m, 2H *Ph*), 7.39 (t, 2H, *Ph*), 7.62 (d, 2H, *Ph*), 7.76 (d, 2H, *Ph*); ^{13}C-NMR ($CDCl_3$): δ 47.69 (Fmoc *CH*), 57.23 (O*CH*$_3$), 60.16 (C-3), 62.02 (C-6), 67.50 (Fmoc *CH*$_2$), 69.28, 72.58, 78.15 (C-2,4,5), 10.27 (C-1), 120.37, 125.70, 127.61, 128.20 (*Ph*), 141.85, 144.59 (C_q, *Ph*), 159.07 (Fmoc *CO*).

Composition **30** (1.65 g) was trityleted with chlorotri-phenylmethane (2.08 g) in pyridine (20 mL), and acetylated with acetic anhydride (1.35 mL) to provide methyl 2,4-di-O-acetyl-6-O-trityl-3-(9-fluorenyl-methoxycarbonyl)amino-3-deoxy-β-D-glucopyranoside (**31**) (2.68 g, 91%), ^{1}H-NMR ($CDCl_3$): δ 1.71, 2.02 (CO*CH*$_3$), 3.04 (dd, H-6b), 3.33 (dd, H-6a), 3.54 (s, 3H, O*CH*$_3$), 3.59 (ddd, 1H, H-5), 4.04 (dd, 1H, H-3), 4.14 (dd, 1H, Fmoc*CH*$_2$), 4.23 (dd, t, 2H, Fmoc *CH*$_2$ and *CH*), 4.58 (d, 1H, H-1 $J_{1,2}$=7.7 Hz), 4.96 (dd, 1H, H-2), 5.17 (t, 1H, H-4), 5.27 (d, 1H, NH), 7.14, 7.25 (m, 12H, *Ph*), 7.34 (t, 2H), 7.46, 7.51, 7.77 (3d, 9H, *Ph*); ^{13}C-NMR ($CDCl_3$): δ 20.46, 20.80 (CO*CH*$_3$), 46.85 (Fmoc *CH*), 55.94 (C-3), 56.35 (O*CH*$_3$), 61.81 (C-6), 67.34 (Fmoc *CH*$_2$), 68.68 (C-4), 71.91 (C-2), 74.29 (C-5), 86.40 (*C*(Ph)$_3$), 101.83 (C-1), 119.90, 125.11, 126.99, 127.11, 127.67, 127.76, 128.66 (*Ph*), 141.16, 143.62, 143.72 (C_q, *Ph*), 156.35 (Fmoc *CO*), 169.78, 170.33 (*COCH*$_3$).

Composition 31 (2.52 g) was oxidized as described previously to provide the title compound (32) (1.61 g, 92%), ^{1}H-NMR ($CDCl_3$): δ 1.87, 1.92 (2s, 6H, $COCH_3$), 3.38 (s, 3H, OCH_3), 3.79 (dd, 1H, H-3), 3.86 (d, 1H, H-5), 4.20 (m, 3H, Fmoc CH_2, CH), 4.51 (d, 1H, H-1 $J_{1,2}$=7.8 Hz), 4.83 (t, 1H, H-2), 5.04 (t, 1H, H-4), 7.32, 7.41 (2t, 4H, *Ph*), 7.48 (d, 1H, NH), 7.63, 7.87 (2d, 4H, *Ph*); ^{13}C-NMR ($CDCl_3$): δ 20.50, 20.69 ($COCH_3$), 46.60 (Fmoc *CH*), 55.07 (C-3), 56.11 (OCH_3), 65.53 (Fmoc CH_2), 70.27, 71.17, 75.28 (C-2,4,5), 101.46 (C-1), 119.98, 125.07, 126.98, 127.54 (*Ph*), 140.63, 143.75 (C_q, *Ph*), 155.82 (Fmoc *CO*), 168.75, 168.83 ($COCH_3$), 170.96 (*COOH*).

Structures of 3-amino-3-deoxy uronic acid derivatives



24	R_1 = Ac	R_2 = N_3	R_3 = CH_2OAc
25	R_1 = H	R_2 = N_3	R_3 = CH_2OH
26	R_1 = Bz	R_2 = N_3	R_3 = CH_2OTr
27	R_1 = Bz	R_2 = N_3	R_3 = CO_2H
28	R_1 = Ac	R_2 = NHFmoc	R_3 = CH_2OAc
30	R_1= H	R_2 = NHFmoc	R_3 = CH_2OH
31	R_1= Ac	R_2 = NHFmoc	R_3 = CH_2OTr
32	R_1 = Ac	R_2 = NHFmoc	R_3 = CO_2H

Example 4

Synthesis of saccharo amino acids - 4-amino-4-deoxy uronic acid derivatives

Methyl 2,3-di-O-acetyl-4-azido-4-deoxy-β-D-glucopyranoside uronic acid (37)

Methyl β-D-galactopyranoside (7.00 g) was dissolved in pyridine (50 mL) and the solution was cooled to -30 °C. Benzoyl chloride was added dropwise to the solution. After conventional work-up methyl 2,3,6-tri-O-benzoyl-β-D-galactopyranoside (**33**) (10.45 g, 56%) was obtained. ^{1}H-NMR ($CDCl_3$): δ 3.52 (OCH_3), 4.12 (t, 1H, H-6), 4.44 (d, 1H, H-4), 4.67 (ddd, dd, 2H, H-5,6), 4.71 (d, 1H, H-1 $J_{1,2}$=8.0 Hz), 5.42 (dd, H-3), 5.91 (dd, 1H, H-2), 7.15-7.60 (m, 10H, COPh), 7.96-8.08 (5H, COPh); ^{13}C-NMR ($CDCl_3$): δ 56.77 (OCH_3), 63.20 (C-6), 67.30, 69.64, 72.49, 74.32 (C-2,3,4,5), 102.15 (C-1), 128.30, 128.36, 128.40, 128.92, 129.47, 129.55, 129.67, 129.72, 129.80, 133.11, 133.23, 133.35 (COPh), 165.53, 166.01, 166.51 (COPh).

Composition **33** (10.25 g) was reacted in pyridine (40 mL) with *p*-toluenesulphonyl chloride (5.72 g), as described earlier, to give Methyl 2,3,6-tri-O-benzoyl-4-O-tosyl-β-D-galactopyranoside (**34**) (10.51 g, 80%), ^{1}H-NMR ($CDCl_3$): δ 2.21 (CH_3 tosyl), 3.45 (OCH_3), 4.24 (dd, ddd, 2H, H-5,6), 4.57 (dd, 1H, H-6), 4.68 (d, 1H, H-1 $J_{1,2}$=7.8 Hz), 5.43 (dd, 1H, H-3), 5.51 (d, 1H, H-4), 5.71 (dd, 1H, H-2), 7.04 (d, 2H, Ph), 7.32 (m, 4H, Ph), 7.46 (m, 4H Ph), 7.56 (t, 1H, Ph), 7.68 (d, 2H, Ph), 7.89, 7.94 (2d, 4H, Ph), 8.07 (d, 2H, Ph); ^{13}C-NMR ($CDCl_3$): δ 21.44 (CH_3 tosyl),

56.93 (OCH_3), 61.88 (C-6), 68.97, 70.91, 71.51, 74.94 (C-2,3,4,5), 102.09 (C-1), 127.40, 128.14, 128.22, 128.40, 129.57, 129.61, 129.89, 133.14, 133.23, 133.28 (*Ph*), 144.80 (C_q CH_3*Ph*), 165.00, 165.69, 165.76 (*CO*Ph).

Composition 34 (9.30 g) was treated in N,N-dimethylformamide (150 mL) with sodium azide (4.55 g). The reaction was worked up as described previously and the product purified by colum chromatography (toluene-ethyl acetate, 9:1→4:1) to provide methyl 4-azido-2,3,6-tri-O-benzoyl-4-deoxy-β-D-glucopyranoside (35) (6.21 g, 83%); ^{1}H-NMR ($CDCl_3$): δ 3.50 (OCH_3), 3.79 (ddd, 1H, H-5), 3.95 (t, 1H,H-4), 4.64 (dd, 1H, H-6a), 4.66 (d, 1H, H-1 $J_{1,2}$=7.7 Hz), 4.77 (dd, 1H, H-6b), 5.43 (dd, 1H, H-2), 5.72 (d, 1H, H-3), 7.36 (m, 5H, *Ph*), 7.50 (m, 4H, *Ph*), 7.60 (t, 1H, *Ph*), 7.94 (m, 4H, *Ph*), 8.12 (dd, 2H, *Ph*); ^{13}C-NMR ($CDCl_3$): δ 57.15 (OCH_3), 63.34 (C-6), 60.93, 71.78, 72.56, 73.73 (C-2,3,4,5), 101.06 (C-1), 128.36, 128.47, 128.54, 129.76, 129.84, 133.28, 133.36, 133.55 (*Ph*), 165.28, 165.66, 166.11 (*CO*Ph).

Composition 35 (6.10 g) was debenzoylated with sodium methoxide in methanol (100 mL) to afford methyl 4-azido-4-deoxy-β-D-glucopyranoside (36) (2.52 g, quant.), ^{1}H-NMR (D_2O): δ 3.36 (dd, 1H, H-2), 3.41 (m, 2H, H-4, 6), 3.55 (OCH_3), 3.62 (t, 1H, H-3), 3.75 (ddd, 1H, H-5), 3.91 (dd, 1H, H-6), 4.38 (d, 1H, H-1 $J_{1,2}$=7.8 Hz); ^{13}C-NMR ($CDCl_3$): δ 57.44 (OCH_3), 61.08 (C-6), 62.00, 73.35, 74.61, 75.34 (C-2,3,4,5), 103.43 (C-1).

Composition 36 (0.88 g) was oxidized in sat. sodium hydrogencarbonate (20 mL) with sodium hypochlorite (27 mL), in the presence of TEMPO (40 mg). After complete conversion of the starting material the reaction mixture

was lyophilized, the residue was taken up in pyridine (10 mL) and was acetylated. The acetylated 4-azido uronic acid was isolated by column chromatography (toluene-10% aq. methanol-aceton, 2:1:1) to give the title compound (37) (0.62 g, 67%), ^{1}H-NMR (CD_3OD): δ 2.02, 2.07 (2s, $COCH_3$), 3.50 (OCH_3), 3.84 (d, 1H, H-5), 4.58 (d, 1H, H-1 $J_{1,2}$=8.0 Hz), 4.90 (dd, t, H-2,4), 5.18 (t, H-3); ^{13}C-NMR (CD_1OD): δ 20.67, 20.72 ($COCH_3$), 57.83 (OCH_3), 63.30, 72.99, 74.68, 76.65 (C-2,3,4,5), 102.79 (C-1), 171.30, 171.42 ($COCH_3$), 174.88 (*C*OOH).

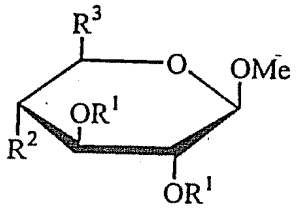
Methyl 2,3-di-O-benzoyl-4-(9-fluorenylmethoxycarbonyl) amino-4-deoxy-β-D-gluco-pyranoside uronic acid (40)

Composition **36** (3.28 g) was hydrogenated in a mixture of methanol water 2:1 (60 mL) in the presence of 10% palladium on activated carbon at atmospheric pressure to provide the free amino-derivative. The catalyst was filtered off and the solvent was evaporated. The crude product was dissolved in water (30 mL), sodium bicarbonate (2.52 g) was added and the mixture was stirred until the solid was dissolved, then it was cooled to 0 °C and a solution of FmocCl (5.04 g) in 1,4-dioxane (40 mL) was added dropwise and the mixture was stirred overnight. It was diluted with chloroform containig 20% of tetrahydrofuran (400 mL) and the organic layer was separated and washed with water. Column chromatography (chloroform-methanol, 9:1) gave methyl 4-(9-fluorenylmethoxycarbonyl)amino-4-deoxy-β-D-glucopyranoside (38) (5.22 g, 84%).

Composition 38 (5.22 g) was converted into the trityl derivative with chlorotriphenylmethane (4.91 g) in pyridine (20 mL), which then was benzoylated with benzoyl chloride (8.75 mL) to provide methyl 2,3-di-O-benzoyl-4-(9-fluorenylmethoxycarbonyl)amino-4-deoxy-6-O-trityl-β-D-glucopyranoside (39) (9.57g, 88%), ^{1}H-NMR ($CDCl_3$): δ 3.40 (bs, 1H, H-6a), 3.58 (s, 3H, OCH_3), 3.83 (t, 1H, Fmoc *CH*), 3.92 (3dd, 3H, H-6b, Fmoc CH_2), 4.06 (m, 2H, H-4,5), 4.74 (d, 1H, H-1 $J_{1,2}$=7.9 Hz), 5.12 (d, 1H, NH), 5.56 (d,t, 2H, H-2, NH), 5.81 (t, 1H, H-3), 6.98-7.38 (m, 18H, *Ph*), 7.48 (m, 9H, *Ph*), 7.62, 7.92, 7.98 (3d, 6H, *Ph*); ^{13}C-NMR ($CDCl_3$): δ 46.86 (Fmoc *CH*), 53.16 (C-4), 56.46 (OCH_3), 63.06, (C-6), 66.88 (Fmoc CH_2), 72.26, 73.11, 74.47 (C-2,3,5), 86.64 ($C(Ph)_3$), 101.63 (C-1), 119.80, 125.00, 125.26, 126.03, 127.54, 127.72, 128.17, 128.25, 128.65, 128.97, 129.39, 129.69, 129.89, 130.02, 133.09, 133.30, 133.35 (*Ph*), 141.00, 141.06, 143.55, 143.78 (C_q, *Ph*), 155.53 (Fmoc *CO*), 165.28, 166.77 (*CO*Ph).

Composition 39 (4.50 g) was oxidized as described previously to give the title product (40) (3.14 g, 95%), ^{1}H-NMR (CD_3OD): δ 3.50 (s, OCH_3), 3.92 (t, 1H, Fmoc *CH*), 4.03, 4.18 (2dd, 2H, Fmoc CH_2), 4.30 (t, 2H, H-4,5), 4.81 (d, 1H, H-1 $J_{1,2}$=8.1 Hz), 5.41 (t, 1H, H-2), 5.79 (m, 1H, H-3), 7.06, 7.13 (t, d, 3H, *Ph*), 7.23 (m, 4H, *Ph*), 7.38 (m, 5H, *Ph*), 7.49 (t, 1H, *Ph*), 7.64 (d, 2H, *Ph*), 7.88 (3d, 3H, *Ph*); ^{13}C-NMR (CD_3 OD): δ 47.83 (Fmoc *CH*), 54.07 (C-4), 57.60 (OCH_3), 68.16 (Fmoc CH_2), 73.61 (C-2), 74.19 (C-3), 75.97 (C-5), 102.87 (C-1), 130.59, 125.93, 126.07, 127.88, 128.45, 129.22, 129.33, 130.41, 130.67, 134.28 (*Ph*), 124.12, 144.61, 144.74, 144.82 (C_q, *Ph*), 158.25 (Fmoc *CO*), 166.59, 167.19 (*CO*Ph), 173.32 (*COOH*).

Structures of 4-amino-4-deoxy uronic acid derivatives



35	R_1 = Bz	R_2 = N_3	R_3 = CH_2OBz
36	R_1 = H	R_2 = N_3	R_3 = CH_2OH
37	R_1 = Ac	R_2 = N_3	R_3 = CO_2H
38	R_1 = H	R_2 = NHFmoc	R_3 = CH_2OH
39	R_1 = Bz	R_2 = NHFmoc	R_3 = CH_2OTr
40	R_1= Bz	R_2 = NHFmoc	R_3 = CO_2H

Example 5

Synthesis of saccharo amino acids - Compounds in which the carboxyl group is not directly attached to the sugar ring

Methoxycarbonylmethyl 6-amino-6-deoxy-β-D-glucopyranoside (45)

Acetobromo glucose (20.56 g) was converted into methoxycarbonylmethyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (41) by reacting the donor in dichloromethane (100 mL) with methyl glycolate (20 mL) in the presence of mercury(II) oxide (1.07 g) and mercury(II) bromide overnight at 0 °C. The reaction mixture was diluted with chloroform, filtered through a pad of Celite, the filtrate was washed with 10% aq. potassium iodide, water, was dried and evaporated. Purification by column

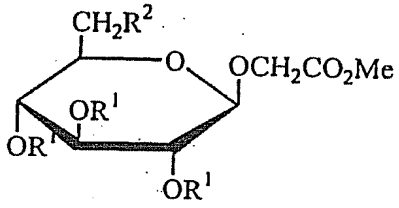
chromatography (toluene-ethyl acetate, 4:1→3:2) provided the protected glycoside (41) (12.97 g, 60%), $[\alpha]_D$ +26.7° (*c* 1.01, chloroform), ^{1}H-NMR ($CDCl_3$): δ 2.01, 2.03, 2.09, 2.10 (4s, $COCH_3$), 3.71 (dd, 1H, H-5), 3.76 (s, $COOCH_3$), 4.14 (dd, 1H, H-6b), 4.27 (dd, 1H, H-6a) 4.31 (bs, 2H, OCH_2), 4.67 (d, 1H, H-1 $J_{1,2}$=7.9 Hz), 5.05 (dd, 1H, H-2), 5.09 (t, 1H, H-4), 5.25 (t, 1H, H-3); ^{13}C-NMR ($CDCl_3$): δ 20.60, 20.72 (4C, $COCH_3$), 51.98 ($COOCH_3$), 61.77 (C-6), 64.59 (OCH_2), 68.29, 70.92, 71.92, 72.50 (C-2,3,4,5), 100.13 (C-1), 169.43, 169.61, 169.65, 170.18, 170.64 ($COCH_3$).

Composition 41 (7.00 g) was deacetylated in methanol (100 mL) by adjusting the pH to 8-9 with sodium methoxide. The solution was neutralized, the solvent was evaporated. The residue was dried *in vacuo*, was dissolved in pyridine (50 mL) and was treated with *p*-toluenesulphonyl chloride (3.40 g) to afford the 6-O-tosyl derivative, which was acetylated with acetic anhydride (10.10 mL). The reaction mixture was poured into ice-water, after conventional work-up, the crude product was recrystallized from ethanol to give crystalline methoxycarbonylmethyl 2,3,4-tri-O-acetyl-6-O-tosyl-β-D-glucopyranoside (42) (7.85 g, 91%), ^{1}H-NMR ($CDCl_3$): δ 2.00, 2.07 (4s, $COCH_3$), 2.45 (s, CH_3 tosyl), 3.74 (dd, 1H, H-5), 3.75 (s, $COOCH_3$), 4.09 (2dd, 2H, H-6), 4.21 (bs, 2H, OCH_2), 4.61 (d, 1H, H-1 $J_{1,2}$= 7.7 Hz), 4.92 (t, 1H, H-4), 4.96 (dd, 1H, H-2), 5.20 (t, 1H, H-3); ^{13}C-NMR ($CDCl_3$): δ 20.53, 20.58, 20.67 (4C, $COCH_3$), 21.68 (CH_3 tosyl), 51.99 ($COOCH_3$); 64.79 (C-6), 67.60 (OCH_2), 68.56, 70.78, 74.71, 72.25 (C-2,3,4,5), 99.87 (C-1), 128.08, 129.95 (Ph *tosyl*), 145.26 (C_q *tosyl*), 169.49, 169.60, 170.10 ($COCH_3$).

Composition 42 This derivative (5.32 g) was converted into the azido derivative using the previously described method to give methoxycarbonylmethyl 2,3,4-tri-O-acetyl-6-azido-6-deoxy-β-D-glucopyranoside (43) (3.43 g, 85%); ^{1}H-NMR ($CDCl_3$): δ 2.00, 2.07 (4s, $COCH_3$), 2.45 (s, CH_3 tosyl), 3.74 (dd, 1H, H-5), 3.75 (s, $COOCH_3$), 4.09 (2dd, 2H, H-6), 4.21 (bs, 2H, OCH_2), 4.61 (d, 1H, H-1 $J_{1,2}$= 7.7 Hz), 4.92 (t, 1H, H-4), 4.96 (dd, 1H, H-2), 5.20 (t, 1H, H-3); ^{13}C-NMR ($CDCl_3$): δ 20.53, 20.58, 20.67 (4C, $COCH_3$), 21.68 (CH_3 tosyl), 51.99 ($COOCH_3$), 64.79 (C-6), 67.60 (OCH_2), 68.56, 70.78, 74.71, 72.25 (C-2,3,4,5), 99.87 (C-1), 128.08, 129.95 (Ph *tosyl*), 145.26 (C_q *tosyl*), 169.49, 169.60, 170.10 ($COCH_3$).

Composition 43 (0.61) g was deacetylated in methanol (10 mL) with sodium methoxide. After complete conversion of the starting material the reaction mixture was neutralized with AG 50W-X8 [H^+] ion-exchange resin and the solvent was evaporated to give 0.41 g (quant.) methoxycarbonylmethyl 6-azido-6-deoxy-β-D-glucopyranoside (44), ^{1}H-NMR (D_2O): δ 3.37 (m, 2H), 3.49 (dd, 1H), 3.56 (m, 3H), 3.77 (s, 3H, CH_3), 4.44 (dd, 2H, OCH_2), 4.53 (d, 1H, H-1 $J_{1,2}$=7.8 Hz); ^{13}C-NMR (D_2O): δ 51.12 (OCH_2COOCH_3), 52.76 (OCH_2COOCH_3), 66.33 (C-6), 70.63, 73.14, 75.31, 75.57 (C-2,3,4,5), 102.44 (C-1), 172.42 (OCH_2COOCH_3) which can be hydrogenolysed in methanol in the presence of 10% palladium on charcoal to give the free amine (45).

Structures of methxycarbonylmethyl D-glucopyranoside derivatives



41	R_1 = Ac	R_2 = OAc
42	R_1 = Ac	R_2 = OTs
43	R_1 = Ac	R_2 = N_3
44	R_1 = H	R_2 = N_3
45	R_1 = H	R_2 = NH_2

Example 6

Synthesis of saccharo amino acids - C-Glycosyl amino acids

3-C-(2,3,4-Tri-O-benzyl-α-L-fucopyranosyl)-N-tert-butoxycarbonyl-alanine ethyl ester (50)

Ethyl 2-(trimethylsilylmethyl)acrylate (15 g) was added to a solution of 1-O- acetyl-2,3,4-tri-O-tribenzyl-L-fucopyranose (16 g) in acetonitrile (100 mL) under nitrogen. The mixture was cooled to 0 °C and to the cooled solution was added boron trifluoride etherate (12 mL). The reaction mixture was stirred overnight and was allowed to come to room temperature slowly. The reaction mixture was cooled to 0 °C, was neutralized with aquous $NaHCO_3$, diluted with dichloromathane (500 mL), washed with water (3x100 mL). The dichloromethane solution was dried over Na_2SO_4 and was concentrated. Column chromatography (hexanes-ethyl acetate, 8:1) of the residue on silica gel

gave ethyl 3-C-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-methacrylate (46) 9.66g, 55%) as a white gum: $[\alpha]_D$ -32.8 (*c* 1.10, chloroform); R_f 0.34 (hexanes-acetone, 5:1); MS: $[M+Na])^+$ 553.4, $[M+H]^+$ 531.3.

A solution of 46 (9.66) in a mixture of dichloromethane-methanol (4:1, 125 mL) was cooled to -78 °C and O_3 was carefully bubbled through the solution until a blue color appeared. The reaction mixture was quenched with dimethyl sulfide (15 mL) and slowly warmed up to room temperature overnight with stirring. The solvents and the excess of dimethyl sulfide were evaporated and the residue was dissolved in CH_2Cl_2 (400 mL), the solution was washed with aqueous $NaHCO_3$, water (2x100 mL), and was dried with Na_2SO_4. After concentration, ethyl 3-C-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-pyruvate (47) (9.5 g, 95%), obtained as a clear oil, was pure enough to be used directly in the next step.

A small amount product was purified by column chromatography and the following analytical data was collected for this purified sample: R_f 0.38 (hexanes-ethyl acetate, 4:1), $[\alpha]_D$ -23.2 (*c* 1.08, chloroform); MS: $[M+Na]^+$ 555.4, [M+Na-NaOBn] 425.4.

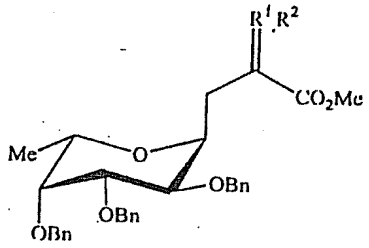
A solution of compound 47 (9.5 g) in ethanol (15 mL) was added to a solution of hydroxylamine chloride (4.6g) in pyridine-ethanol (1:1, 100 mL) at room temperature. The reaction mixture was stirred for 1 hour, then it was poured into ice-water (200 mL), was stirred for 10 minutes, and extracted with dichloromethane (3x150 mL). The combined CH_2Cl_2 solution was dried with Na_2SO_4 and concentrated to give Ethyl 3-C-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-pyruvate oxime (48) as a clear oil (9.85 g,

98%). A small amount of the crude product (0.64 g) was purified by column chromatography (toluene-acetone, 9:1). Two fractions were collected and they were proved to be the *E* and *Z* oxime isomers by NMR. Isomer-1: 0.030 g, R_f 0.5 (toluene-acetone, 8:1); $[\alpha]_D$ -36.1 (*c* 1.0, chloroform); FAB-MS: $[M+Na]^+$ 570.4, $[M+H]^+$ 548.4. Isomer-2: 0.550 g; R_f 0.38 (toluene-acetone, 8:1); $[\alpha]_D$ -43.1, (*c* 1.7, chloroform); MS: same as that of isomer-1.

A solution of **48** (8.7 g) in ethanol (30 mL) was hydrogenated in the presence of Raney nickel at room temperature for two days. TLC (toluene-acetone, 3:1) showed the absence of the starting material and the appearance of two new spots (R_f 0.39 and R_f 0.32) in an about 1:1 ratio. The solids were removed by filtration through Celite and were washed with ethanol. The combined filtrate and washings were concentrated to give 3-C-(2,3,4-Tri-O-benzyl-α-L-fucopyranosyl)-alanine ethyl ester (**49**) (7.06 g, 84%) as a clear oil, which was used directly in the next step.

To a solution of **49** (7.06 g) in ethanol (40 mL) was added an excess of di-*tert*-butyl dicarbonate at room temperature. The reaction mixture was stirred for 1 hour, then was evaporated to dryness. The residue was purified by column chromatography (hexanes-ether, 3:1) to give the D and L stereoisomers of **50**. Combined yield: 7.16 g, 80%. Isomer-1: 2.5 g, R_f 0.28 (hexanes-ether, 2:1); $[\alpha]_D$ -25.3 (*c* 1.25, chloroform); FAB-MS: $[M+H]^+$ 635.5, [M-NHBoc] 535.6, $[M-H]^-$ 632.1, $[M-CO_2CH_2CH_3]$ 558.2. Isomer-2: 3.2 g, R_f 0.25 (hexanes-ether, 2:1); $[\alpha]_D$ -18.7 (*c* 1.25, chloroform); FAB-MS: same as that of isomer-1.

Structures of C-glycosyl amino acid derivatives



46 R_1, R_2 = $=CH_2$

47 R_1, R_2 = $=O$

48 R_1, R_2 = $=NHOH$

49 R_1, R_2 = H, NH_2

50 R_1, R_2 = H, NHBoc

Example 7

Synthesis of saccharopeptides - (1→6) Linked homooligomers

N-(Methyl 2,3,4-tri-O-acetyl-D-glucopyran-1-osyl uronate)-(1-azido-1-deoxy-2,3,4-tri-O-acetyl-β-D-glucopyranuronamide) (51α and 51ß)

(a) Compositions 2 (0.432 g) and 6 (403 mg) were dissolved in DMF (5 ml) and N-isobutoxycarbonyl-2-isobutoxy-1,2-dihydroquinoline (IIDQ) (385 µl) was added drop-wise into the solution. The reaction mixture was stirred at room temperature until the free carboxylic acid derivative was consumed. The solvent was evaporated and the residue was purified by column chromatography (toluene-acetone, 9:1 85:15) to yield N-{Methyl 2,3,4-tri-O-acetyl-ß-D-glucopyran-1-osyl uronate}-{1-azido-1-deoxy-2,3,4-tri-O-acetyl-ß-D-glucopyranuronamide} (51ß, 248 mg, 31%). $[\alpha]_D$ -8.7° (*c* 0.98, chloroform); ^{1}H-NMR data ($CDCl_3$): δ 2.02, 2.04, 2.05, 2.09, 2.16 (5s, 18H, $COCH_3$), 3.73 (s, 3H, $COOCH_3$), 3.98, 4.13 (2d, 2H, H-5,5'), 4.68 (d, 1H, H-1, $J_{1,2}$ = 8.9 Hz), 4.94 (t, 1H), 4.95 (t, 1H), 5.00 (t, 1H), 5.13 (d, 1H, H-1', $J_{1',2'}$ = 9.5 Hz), 5.18 (t, 1H), 5.41 (t, 1H), 7.34 (d, 1H, NH). ^{13}C-NMR data ($CDCl_3$): δ 20.4, 20.5, 20.5, 20.6 (6C, $COCH_3$), 52.9 ($COOCH_3$), 68.8, 69.6, 70.0, 70.5, 71.7, 71.7, 73.0, 73.9 (8C, C-2,3,4,5,2',3',4',5'), 77.6 (C-1'), 88.0 (C-1), 166.7, 167.2 (2C, CONH and $COOCH_3$), 169.3, 169.5, 169.6, 169.6, 169.8, 171.2 (6C, $COCH_3$).

The α isomer, N-{methyl 2,3,4-tri-O-acetyl-α-D-glucopyran-1-osyl uronate}-{1-azido-1-deoxy-2,3,4-tri-O-acetyl-β-D-glucopyranuronamide} (51α) eluted second (248

mg, 31%). $[\alpha]_D$ +6.0° (*c* 1.00, chloroform); ^{1}H-NMR data (CD_3OD): δ 1.97, 1.98, 2.03, 2.04, 2.06 (5s, 18H, $COCH_3$), 3.72 (s, 3H, $COOCH_3$), 4.28 (d, 1H), 4.45 (d, 1H, H-1, $J_{1,2}$ = 8.6 Hz), 5.00-5.07 (m, 2H), 5.07 (t, 1H), 5.12 (t, 1H), 5.32 (d, 1H), 5.34 (d, 1H), 5.67 (t, 1H), 5.96 (d, 1H, H-1', $J_{1',2'}$ = 5.1 Hz). ^{13}C-NMR data (CD_3OD): δ 20.5, 20.5, 20.5, 20.6 (6C, $COCH_3$), 53.1 ($COOCH_3$), 69.7, 70.0, 70.2, 70.3, 71.2, 71.7, 73.5, 74.6, 76.6 (9C, C-2,3,4,5,1',2',3',4',5'), 88.9 (C-1), 169.1 (CONH and $COOCH_3$), 170.7, 171.0, 171.1, 171.2 (6C, $COCH_3$).

(b) Coupling of 2 and 6 using N,N-diisopropyl-carbodiimide (DIC) instead of IIDQ afforded 51α and 51ß in a better combined yield (592 mg, 74%) in the same ratio as above.

(c) Coupling of 2 and 6 using THF as a solvent instead of DMF afforded 51ß exclusively (Yield 87%).

N-(Methyl-D-glucopyran-1-osyl uronate)-(1-azido-1-deoxy-β-D-glucopyranuron-amide) (52α and 52ß)

To a solution of 51ß (66 mg) in methanol (5 ml) 1 M methanolic sodium methoxide (0.2 ml) was added and the solution stirred at 0-5°C. The deacetylated product crystallized from the solution. The product was filtered off and washed with cold methanol to furnish N-(methyl β-D-glucopyran-1-osyl uronate)-(1-azido-1-deoxy-β-D-glucopyranuronamide) (52ß, 39 mg, 96%). ^{1}H-NMR data (D_2O): δ 3.33 (t, 1H, H-2), 3.51-3.64 (m, 5H, H-3,4,2',3',4'), 3.82 ($COOCH_3$), 4.04, 4.18 (2d, 2H, H-5,5'), 4.85 (d, 1H, H-1, $J_{1,2}$ = 8.8 Hz), 5.13 (d, 1H, H-1', $J_{1',2'}$ = 8.7 Hz). ^{13}C-NMR data (D_2O): δ 53.4 ($COOCH_3$), 71.2, 71.3, 71.4, 72.5,

75.4, 76.1, 76.3, 76.9 (8C, C-2,3,4,5, 2',3',4',5'), 79.4 (C-1'), 90.4 (C-1), 171.1, 171.5 (2C, $COOCH_3$, NHCO).

Composition 51α was deacetylated as described above. The reaction mixture was neutralized with AG 50W-X8 (H^+) ion-exchange resin. The resin was filtered off and the solvent was evaporated to give N-(methyl α-D-glucopyran-1-osyl uronate)-(1-azido-1-deoxy-β-D-glucopyranuronamide) (52α , 38 mg, 95%). $[\alpha]_D$ +2.8 (c 0.96 H_2O); ^{1}H-NMR data (D_2O): δ 3.84 (t, 1H, H-2), 3.55-3.75 (m, 3H), 3.81 (s, 3H, $COOCH_3$), 3.84-3.91 (m, 2H), 4.13, 4.21 (d, 2H, H-5,5'), 4.85 (d, 1H, H-1, $J_{1,2}$ = 8.7 Hz), 5.73 (d, 1H, H-1', $J_{1',2'}$ = 4.0 Hz). ^{13}C-NMR data (D_2O): δ 53.3 ($COOCH_3$); 68.9, 70.1, 70.1, 71.9, 72.6, 72.8, 75.5, 76.4, 76.7 (C-2,3,4,5,1',2',3',4',5'), 90.4 (C-1), 171.49, 171.51 (2C, $COCH_3$, NHCO).

N-(D-glucopyran-1-osyl uronic acid)-1-azido-1-deoxy-β-D-glucopyranuronamide (53α and 53ß)

The deacetylated product 52ß (32 mg) was dissolved in 0.1 M NaOH (2 ml) and kept overnight at 0-5°C. The solution was neutralized with AG 50W-X8 (H^+) ion-exchange resin, was filtered, and the filtrate was lyophilized to give N-(β-D-glucopyran-1-osyl uronic acid)-1-azido-1-deoxy-β-D-glucopyranuronamide (53ß, 30 mg, 97%). ^{1}H-NMR data (D_2O): δ 3.32 (t, 1H, H-2), 3.50-3.62 (m, 5H), 4.03, 4.09 (2d, 2H, H-5,5'), 5.12 (d, 1H, H-1', $J_{1',2'}$ = 8.8 Hz), the H-1 signal was covered by the signal of HOD. ^{13}C-NMR data (D_2O): δ 71.2, 71.3, 71.4, 72.5, 76.2, 76.3 (C-2,3,4,5,2',3',4',5'), 79.2 (C-1'), 90.4 (C-1), 171.5 (NHCO), 172.7 (COOH).

The deacetylated product 52α (32 mg) was treated in the same way as described above to give N-(α-D-glucopyran-1-osyl uronic acid)-1-azido-1-deoxy-β-D-glucopyranuronamide (53α, 32 mg, 100%). ^{1}H-NMR data (D_2O): δ 3,34 (t, 1H, H-2), 3.55-3.63 (m, 2H), 3.70 (t, 1H), 3.81-3.89 (m, 2H), 4.13, 4.14 (d, 2H, H-5,5'), 4.84 (d, 1H, H-1, $J_{1,2}$=8.9 Hz), 5.73 (d, 1H, H-1' $J_{1',2'}$=4.4 Hz); ^{13}C-NMR data (D_2O): δ 69.0, 71.0, 72.1, 72.5, 72.6, 75.5, 76.4, 76.7 (C-2,3,4,5,1',2',3',4',5'), 90.5 (C-1), 171.5 (NHCO), 172.7 (COOH).

N-(Methyl 2,3,4-tri-O-acetyl-β-D-glucopyran-1-osyl uronate)-N-(2,3,4-tri-O-acetyl-β-D-glucopyran-1-osyl uronamide)-(1-azido-1-deoxy-2,3,4-tri-O-acetyl-β-D-glucopyran-1-osyl uronamide) (55)

A solution of 51β (0.50 g) in 10 mL ethyl acetate was hydrogenated in the presence of 150 mg 10% palladium on charcoal at room temperature and atmospheric pressure to give the amine (54), $[\alpha]_D$ +23.1° (*c* 0.32 chloroform), ^{1}H-NMR ($CDCl_3$) δ 2.01, 2.03, 2.04, 2.07, 2.16 (5s, 18H, $COCH_3$), 3.72 (s, 3H, $COOCH_3$), 3.88 (d, 1H, H-5' *J*=10.0 Hz), 4.13 (d, 1H, H-5 *J*=10.3 Hz), 4.25 (d, 1H, H-1 $J_{1,2}$=8.9 Hz), 4.83 (t, 1H), 4.86 (t, 1H, H-2), 4.98 (t, 1H), 5.13 (t, 1H, m, 2H, NH_2), 5.20 (t, 1H, exchangeable with deuterium and d, 1H, H-1' $J_{1',2'}$=9.9 Hz), 5.29 (t, 1H), 5.40 (t, 1H) 7.33 (d, 1H, *NH* $J_{1'}$,NH=9.6 Hz), ^{13}C-NMR ($CDCl_3$) δ 20.48, 20.59, 20.62, 20.65, 20.79 (6C, $COCH_3$), 52.96 (COO CH_3), 69.61, 69.91, 71.67, 71.86, 72.11, 73.09, 73.96, 76.63, 77.05, 77.47 (C-1',2,3, 4,5,2',3',4',5'),

85.24 (C-1), 167.10, 167.80, 169.56, 169.60, 169.78, 170.19, 171.27 (*CO*NH, 6C, CO*CH*$_3$, COO*CH*$_3$).

Composition 54 and 6 (0.26 g) were dissolved in THF (10 mL), and HOBT (0.25 g) and DIC (0.25 mL) were added into the solution. The reaction mixture was worked up as described earlier, and purified by colum chromatography (toluene-aceton 3:2) to give the title compound (55), 0.62 g, 87 %, $[\alpha]_D$ +1.1° (*c* 1.09 chloroform), ^{1}H-NMR ($CDCl_3$) δ 2.02, 2.03, 2.04, 2.05, 2.11, 2.14, 2.16 (9s, 27H, CO*CH*$_3$), 3.72 (s, 3H, COO*CH*$_3$), 4.00, 4.07, 4.13 (3d, 3H, H-5, H-5', H-5"), 4.83 (d, 1H, H-1 $J_{1,2}$=8.9 Hz), 4.90 (t, 1H), 4.95 (t, 1H), 4.97 (t, 1H), 4.98 (t, 1H), 5.00 (t, 1H), 5.13 (t, 1H, H-4), 5.20, 5.21 (2t, 2H, H-1', H1" $J_{1,2}$=9.6, $J_{1,2}$=9.6Hz respectively), 5.32 (t, 1H), 5.37 (t, 1H), 5.39 (t, 1H), 7.26, 7.38 (2d, 2H, *NH* $J_{1',NH}$=9.7, $J_{1",NH}$=9.2 Hz, respectively), ^{13}C-NMR ($CDCl_3$) δ 20.47, 20.54, 20.58, 20.62, 20.67 (9C, CO*CH*$_3$), 52.98 (COO*CH*$_3$), 68.62, 69.13, 69.55, 70.02, 70.56, 71.50, 71.63, 71.69 (C-2,3,4,2',3',4',2",3",4"), 73.72, 74.00 (3C, C-5,5',5"), 77.42, 77.46 (C-1',1"), 88.16 (C-1), 166.52, 166.90, 167.07, 169.29, 169.24, 169.55, 169.60, 169.69, 169.77, 171.26, 171.32 (12C, 9 CO*CH*$_3$, 2 *CO*NH, COO*CH*$_3$), FAB MS $[M+H]^+$ 962.6, $[M-H]^-$ 960.6.

N-(Methyl β-D-glucopyran-1-osyl uronate)-N-(β-D-glucopyran-1-osyl uronami-de)-(1-azido-1-deoxy-β-D-glucopyran-1-osyl uronamide) (56)

Composition 55 (0.19 g) was deacetylated in methanol (15 mL) with catalytic amount of 1 M sodium methoxide to give the title product (56) 0.10 g (90%), $[\alpha]_D$ -62.1 (*c*

1.05 water), ^{1}H-NMR (D_2O) δ 3.33 (t, 1H, H-2), 3.51-3.65 (m, 8H, H-3,4,2',3', 4',2",3",4"), 3.81 ($COOCH_3$), 4.05, 4.16 (3d, 3H, H-5, H-5',5"), 4.85 (d, 1H, H-1 $J_{1,2}$=9 Hz), 5.11, 5.14 (2d, 2H, H-1',H" $J_{1,2}$=8.8, $J_{1,2}$=8.8 Hz, respectively), ^{13}C-NMR (D_2O) δ 53.36 ($COOCH_3$), 71.27, 71.35, 72.52, 75.43, 76.08, 76.28, 76.95 (12C, C-2,3,4,5,2',3',4',5',2",3",4",5"), 79.32, 79.39 (C-1',1"), 90.45 (C-1), 171.09, 171.53, 171.73 (3C, $COOCH_3$, CONH).

N-(β-D-glucopyran-1-osyl uronate)-N-(β-D-glucopyran-1-osyl uronamide)-(1-azido-1-deoxy-β-D-glucopyran-1-osyl uronamide) (57)

Composition 56 (83 mg) was treated with 0.1 M NaOH (2 mL) at 0 °C for one minute to obtain the free uronic acid (57), 80 mg (99%), ^{1}H-NMR (D_2O) δ 3.32 (t, 1H, H-2), 3.50-3.66 (m, 8H), 4.04, 4.07, 4.11 (3d, 3H, H-5, H-5',5"), 4.85 (d, 1H, H-1' $J_{1',2'}$=8.8 Hz), 5.11, 5.14 (2d, 2H, H-1',1" $J_{1,2}$=8.8, $J_{1,2}$=8.9 Hz, respectively), ^{13}C NMR (D_2O) δ 71.28, 71.38, 71.41, 72.54, 75.45, 76.10, 76.18, 76.20, 76.97 (C-2,3,4, 5,2',3',4',5',2",3",4", 5"), 79.28, 79.33 (C-1',1"), 90.45 (C-1), 171.52, 171.72 (CONH), 172.41 (COOH).

N-(Methyl 2,3,4-tri-O-acetyl-β-D-glucopyran-1-osyl uronate)-N-(2,3,4-tri-O-acetyl-β-D-glucopyran-1-osyl uronamide)-N-(2,3,4-tri-O-acetyl-β-D-glucopyran-1-osyl uronamide)-(1-azido-1-deoxy-2,3,4-tri-O-acetyl-β-D-glucopyran-1-osyl uronamide) (58)

Composition 55 (0.20 g) was hydrogenated in ethyl acetate (10 mL), in the presence of 10 % palladium on carbon (0.15 g) to give the protected amine, which was coupled to 6 (80 mg) in THF (5 mL) with DIC (60 mL) to give the protected tetramer (58) (0.21 g, 80 %), ^{1}H-NMR ($CDCl_3$) δ 2.01, 2.02, 2.04, 2.05, 2.07, 2.09, 2.11, 2.16 (12s, 36H, $COCH_3$), 3.72 (s, 3H, $COOCH_3$), 3.96, 4.06, 4.10 (4d, 4H, H-5, H-5', H-5",5"'), 4.77 (d, 1H, H-1 $J_{1,2}$=8.8 Hz), 4.90 (t, 1H), 4.94-5.51 (m, 7H), 5.12-5.23 (3t, 3H, H-1',1",1"), 5.26-5.42 (m, 4H), 7.49 (3d, 3H, *NH*), ^{13}C-NMR ($CDCl_3$) δ 20.50, 20.55, 20.61, 20.66, 20.69 (12C, $COCH_3$), 52.94 ($COOCH_3$), 68.64, 68.96, 69.11, 69.47, 70.09, 70.14, 70.16, 70.54, 71.65, 71.71, 71.91 (C-2,3,4,2',3',4',2",3",4",2"',3"',4"', 5"'), 73.70, 73.77, 74.05, 74.11 (3C, C-5,5',5",5"'), 77.45, 77.58, 77.78 (C-1',1", 1"'), 87.97 (C-1), 166.75, 166.91, 169.26, 169.48, 169.51, 169.66, 169.79, 171.21, 171.37 (16C, 12 $COCH_3$, 3 *CONH*, $COOCH_3$), FAB MS $[M+H]^+$ 1263.9, $[M-H]^-$ 1261.9.

N-(Methyl β-D-glucopyran-1-osyl uronate)-N-(β-D-glucopyran-1-osyl uron-amide)-N-(β-D-glucopyran-1-osyl uronamide)-(1-azido-1-deoxy-β-D-glucopyran-1-osyl uronamide) (59)

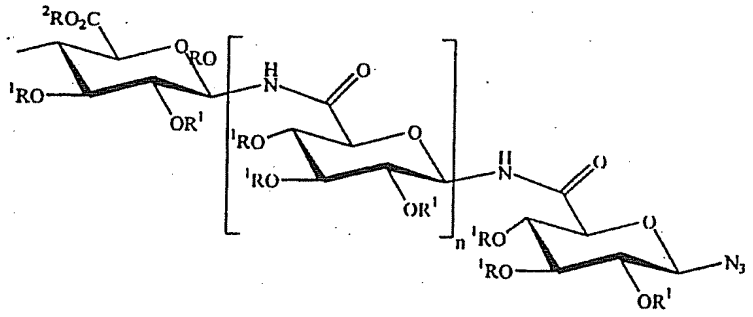
Composition 58 (0.21 g) was deacetylated with 1 M sodium-methoxide in methanol (10 mL) to give 0.11 g (92%) of 59, ^{1}H-NMR (DMSO-d_6): δ 3.33 (t, 1H, H-2), 3.47-3.65 (m, 10H, H-3,4,2',3',4',2",3",4",2"',3"', 4"'), 3.80 ($COOCH_3$), 4.05, 4.15 (4d, 4H, H-5, H-5',5",5"'), 4.84 (d, 1H, H-1 $J_{1,2}$=8.8 Hz), 5.09, 5.11, 5.14 (3d, 3H, H-1',H",H"' $J_{1,2}$=8.8, $J_{1,2}$=8.8, $J_{1,2}$=8.7 Hz, respectively), ^{13}C-NMR (D_2O): δ

53.34 ($COOCH_3$), 71.29, 71.37, 72.53, 75.45, 76.08, 76.12, 76.31, 76.91, 76.97, 77.00 (16C, C-2,3,4,5,2',3',4',5',2",3",4",5",2"',3"', 4"',5"'), 79.36, 79.39, 79.40 (C-1',1",1"'), 90.46 (C-1), 171.08, 171.46, 171.68, 171.71 (4C, $COOCH_3$, *CONH*).

N-(β-D-glucopyran-1-osyl uronate)-N-(β-D-glucopyran-1-osyl uron-amide)-N-(β-D-glucopyran-1-osyl uronamide)-(1-azido-1-deoxy-β-D-glucopyran-1-osyl uronamide) (60)

Composition 59 (0.11 g) was dissolved in 1 M NaOH (2 mL) and kept at 0 °C for one minute. The solution was neutralized with AG-50W-X8 (H^+) ion-exchange resin, and liophilized to give the unprotected tetramer (60) quantitatively, ^{1}H-NMR (D_2O) δ 3.32 (t, 1H, H-2), 3.50-3.66 (m, 8H), 4.04, 4.07, 4.11 (3d, 3H, H-5, H-5',5"), 4.85 (d, 1H, H-1' $J_{1',2'}$=8.8 Hz), 5.11, 5.14 (2d, 2H, H-1',1" $J_{1,2}$=8.8, $J_{1,2}$=8.9 Hz, respectively), ^{13}C NMR (D_2O) δ 71.28, 71.38, 71.41, 72.54, 75.45, 76.10, 76.18, 76.20, 76.97, (C-2,3,4,5,2',3',4',5',2",3",4", 5"), 79.28, 79.33 (C-1',1"), 90.45 (C-1), 171.52, 171.72 (*CONH*), 172.41 (*COOH*).

Structures of (1→6) linked homooligomer saccharopeptides



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52b	n = 0	R_1 = H	R_2 = Me
53b	n = 0	R_1 = H	R_2 = H
55	n = 1	R_1 = Ac	R_2 = Me
56	n = 1	R_1 = H	R_2 = Me
57	n = 1	R_2 = H	R_3 = H
58	n = 2	R_1 = Ac	R_2 = Me
59	n = 2	R_1 = H	R_2 = Me
60	n = 2	R_2 = H	R_3 = H

Example 8

Synthesis of saccharopeptides - (2→6) Linked homooligomers

Methyl{methyl 3,4-di-O-acetyl-2-deoxy-2-[(methyl 3,4-di-O-acetyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside) uronamido]-α-D-glucopyranoside} uronate (61)

(a) Compositions 12 (157 mg) and 10 (216 mg) were dissolved in THF (5 ml) and 1-(3-dimethylaminopropyl)-3-

ethylcarbodiimide hydrochloride (DEC) (95 mg) and N-hydroxybenztriazole (HOBT) (6 mg) were added. The reaction mixture was stirred overnight at room temperature. The mixture was diluted with water and chloroform (20 ml), the organic layer was separated and was washed with water (2 x 5 ml) and evaporated. Column chromatography (toluene-acetone, 3:2) gave composition 61 (149 mg, 41 %). ^{1}H-NMR data ($CDCl_3$): δ 1.88, 1.97, 2.01, 2.03 (4s, 12H, $COCH_3$), 3.37, 3.50 (2s, 6H, 2 OCH_3), 3.74 (s, 3H, $COOCH_3$), 4.05 (m, 2H, H-2,5), 4.28 (m, 2H, H-2',5'), 4.83 (d, 1H, H-1, $J_{1,2}$ = 3.6 Hz), 4.85 (d, 1H, H-1', $J_{1',2'}$ = 3.6 Hz), 5.01 (d, 1H, OCH_2), 5.14 (d, 1H, OCH_2), 5.00-5.15 (m, 2H, H-3,4), 5.18-5.31 (m, 2H, H-3,4'), 5.33 (d, 1H, $NHCOO-CH_2Ph$), 6.61 (d, 1H, NHCO-amide). ^{13}C-NMR data ($CDCl_3$): δ 20.5, 20.5, 20.6, 20.7 (4C, $COCH_3$), 51.2 (C-2'), 52.8 ($COOCH_3$), 53.8 (C-2), 55.7 (OCH_3), 56.1 (OCH_3), 67.0 (OCH_2), 68.5 (C-5'), 68.6 (C-5), 69.3, 69.6, 70.0, 70.1 (C-3,4,3',4'), 98.1 (C-1'), 98.6 (C-1), 128.1, 128.3, 128.5 (5C, aromatic carbons), 136.2 (quaternary aromatic carbon), 155.8 ($OCOCH_2Ph$), 167.5 (NHCO-amide), 168.2 ($COOCH_3$), 169.4, 169.8, 170.7, 170.8 (4C, $COCH_3$).

(b) Coupling of 12 and 10 using N,N-diisopropyl-carbodiimide (DIC) instead of DEC afforded 15 in a better yield (95%).

Methyl {methyl 2-deoxy-2-[(methyl 2-benzyloxycarbonyl-amino-2-deoxy-α-D-glucopyranoside uronamido)-α-D-glucopyranoside]} uronate (62)

Disaccharopeptide 61 (0.11 g) was dissolved in methanol (10 mL), and deacetylated with catalytic amount of sodium methoxide, to give 62 (0.08g, 98%), $[\alpha]_D$ +4.04°

(c 0.97 DMSO), ^{1}H-NMR (DMSO-d_6 + D_2O) δ 3.41 (s, 6H, OCH_3), 3.82 (s, 3H, $COOCH_3$), 4.05 (dd, 2H, H-5, 5'), 4.78, 4.84 (2d, 2H, H-1,1' $J_{1,2}$=3.5, 3.5 respectively), 7.50 (s, 4H, *Ph*), ^{13}C-NMR (DMSO+D_2O) δ 52.45, 53.81, 55.44, 55.62, 55.69 (2x OCH_3, $COOCH_3$, C-2,2'), 65.81 (OCH_2), 70.04, 70.31, 71.69, 72.02, 72.13, 72.44 (C-3,4,5,3',4',5'), 98.66, 98.96 (C-1,1' respectively), 128.11, 128.21, 128.74 (*Ph*), 137.27 (C_q*Ph*), 156.58 ($COOCH_2$Ph), 169.90, 170.14 (*CO*NH, $COOCH_3$).

Methyl 2-deoxy-2-(methyl 2-benzyloxycarbonyl-amino-2-deoxy-α-D-glucopyran-oside uronamido)-α-D-glucopyranoside uronic acid (63)

Composition **62** (80 mg) was treated with 1 M sodium hydroxide to give the free acid (**63**), 74 mg (100%), ^{1}H-NMR (CD_3OD+D_2O) δ 2.98 (s, 6H, OCH_3), 4.38, 4.41 (2d, H-1,1'), 7.08 (s, 4H, *Ph*), ^{13}C-NMR (CD_3OD+D_2O) δ 47.00, 47.05, 47.19, 48.08 (OCH_3, C-2,2'), 55.63 (OCH_2), 91.03, 92.19 (C-1,1'), 119.06, 120.24 (*Ph*), 168.90, 168.97, 168.99 (2 *CO*NH , *CO*OH).

Methyl 2-deoxy-2-(methyl 2-amino-2-deoxy-α-D-glucopyranoside uronamido)-α-D-glucopyranoside uronic acid (64)

Composition **63** (74 mg) was hydrogenolyzed in acetic acid (5 mL) in the presence of 10 % palladium on charcoal (50 mg) to give **64** (55 mg, 100%), ^{1}H-NMR (CD_3OD+D_2O) δ 3.40, 3.44 (2s, 6H, OCH_3), ^{13}C-NMR (CD_3OD+D_2O) δ 53.63,

53.92, 55.94, 55.99, (OCH_3, C-2,2'), 96.93, 98.55 (C-1,1'), 174.92 (*COOH*).

Methyl {methyl 3,4-di-O-acetyl-2-deoxy-2-[methyl 3,4-di-O-acetyl-2-deoxy-2-(methyl 3,4-di-O-acetyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside uronamido)-α-D-glucopyranoside uronamido]-α-D-glucopyranoside} uronate (66)

A solution of 61 (430 mg) in EtOAc (15 ml) was hydrogenated in the presence of 10% Pd-C (150 mg) at room temperature and at atmospheric pressure for one hour. The catalyst was filtered off and the filtrate was evaporated to give methyl{methyl 3,4-di-O-acetyl-2-deoxy-2-[(methyl 3,4-di-O-acetyl-amino-2-deoxy-α-D-glucopyranoside) uronamido]- α-D-glucopyranoside} uronate (65) (350 mg, 100%) and directly used for the coupling reaction. Compositions 65 (350 mg) and 12 (260 mg) were dissolved in THF (10 ml) and 1,3-diisopropylcarbodiimide (0.1 ml) and HOBT (50 mg) were added into the solution. The reaction mixture was stirred for two days at room temperature. The reaction mixture was worked up as described for 61. Column chromatography gave the title product 66 (560 mg, 93%). $[\alpha]_D$ +133.9 (c 1.00 chloroform); ^{1}H-NMR ($CDCl_3$) δ 1.89, 1.97, 2.00, 2.03, 2.04, 2.06 (6s, 18H, $COCH_3$), 3.38, 3.46, 3.51 (3s, 9H, OCH_3), 3.76 (s, 3H, $COOCH_3$), 4.01-4.11 (m, 3H, 2 x H-2 and H-5), 4.18-4.32 (m, 3H, 2 x H-5 and H-2), 4.84 (d, 3H, H-1,1',1", $J_{1,2}$=3.4 Hz), 5.00-5.22 (m, 5H), 5.24-5.35 (m, 4H, incl NH and OCH_2Ph), 6.62 (d, 1H, NH), 6.68 (d, 1H, NH), 7.34 (m, 5H, Ph); ^{13}C-NMR ($CDCl_3$) δ 20.5, 20.5, 20.6, 20.7 (6C, $COCH_3$), 51.2, 51.5, 52.8, 53.7, 55.8, 55.8, 56.1 (C-2,2',2", 3 x OCH_3,

$COOCH_3$), 67.0 (OCH_2Ph), 68.4 (2C), 68.8, 69.3, 69.4, 69.6, 69.7, 69.9, 70.1 (C-3,4,5,3',4', 5',3",4",5"), 97.8, 98.1, 98.5 (C-1,1',1"), 128.1, 128.2, 128.5 (Ph), 136.2 (q Ph), 155.8 ($OCOCH_2Ph$), 167.6, 167.7 (NHCO-amide), 168.2 ($COOCH_3$), 169.4, 169.8, 169.9, 170.7 (6C, $COCH_3$).

Methyl {methyl 2-deoxy-2-[methyl 2-deoxy-2-(methyl 2-benzyloxycarbonyl-amino-2-deoxy-α-D-glucopyranoside uronamido)-α-D-glucopyranoside uronamido]-α-D-glucopyranoside} uronate (67)

Composition 66 (0.197 g) was deacetylated as previously described to give 0.140 g (95%) of 67, ^{1}H-NMR (DMSO-d_6+D_2O) δ 3.41, 3.42 (2s, 9H, OCH_3), 3.66 (s, 3H, $COOCH_3$), 4.20 (3dd, 3H, H-5, 5',5"), 4.66, 4.70 (3d, 3H, H-1,1',1"), 7.47 (s, 4H, *Ph*), ^{13}C-NMR (DMSO-d_6+D_2O) δ 53.85, 55.53, 55.57, 55.76, 55.93 (3x OCH_3, $COOCH_3$, C-2,2',2"), 60.55 (OCH_2), 98.58, 98.72 (3C, C-1,1',1").

Methyl /methyl 3,4-di-O-acetyl-2-deoxy-{methyl 3,4-di-O-acetyl-2-deoxy-2-[methyl 3,4-di-O-acetyl-2-deoxy-2-(methyl 3,4-di-O-acetyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside uronamido)-α-D-glucopyranoside uronamido]-α-D-glucopyranoside uronamido}-α-D-glucopyranoside/ uronate (68)

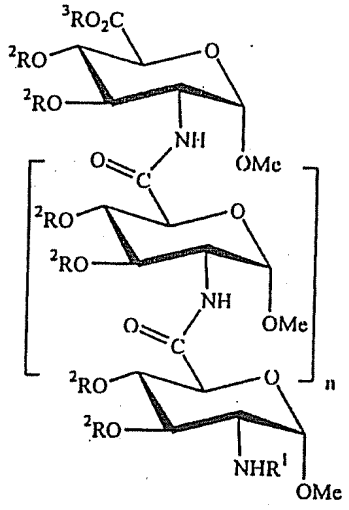
A solution of 66 (320 mg) was hydrogenated as described above, and the free amine (270 mg) was coupled with 12 (130 mg) in a mixture of THF - 1,4-dioxane as described above, to give the title composition 68 (280 mg, 72%). $[\alpha]_D$ +140.9 (c 1.08 chloroform); ^{1}H-NMR ($CDCl_3$) δ 1.90, 1.98, 1.99, 2.03, 2.04, 2.06, 2.08 (8s, 24H,

$COCH_3$), 3.38, 3.45, 3.49, 3.52 (4s, 24H, OCH_3), 3.76 (s, 3H, $COOCH_3$), 4.03-4.12 (m, 4H), 4.19-4.32 (m, 4H), 4.82-4.86 (2d, 4H, H-1,1',1",1'''), 4.98-5.35 (m, 11H, 8 sugar skeleton, NH and OCH_2Ph), 6.62, 6.67, 6.69 (3d, 3H, NH), 7.34 (d, 5H, Ph); ^{13}C-NMR ($CDCl_3$) δ 20.5, 20.6, 20.6, 20.7, 20.7 (8C, $COCH_3$), 51.1, 51.5, 52.9, 53.7, 55.7, 55.8, 56.1 (9C, C-2,2',2",2''', 4 x OCH_3, $COOCH_3$), 67.1 (OCH_2Ph), 68.3, 68.4, 68.6, 69.3, 69.5, 69.5, 69.6, 69.9, 70.1 (12C, C-3,4,5,3',4', 5',3",4",5",3''',4''',5'''), 97.0, 98.1, 98.5 (4C, C-1,1',1'',1'''), 128.1, 128.3, 128.53 (Ph), 136.1 (q Ph), 155.8 ($OCOCH_2Ph$), 167.6, 167.7, 167.8 (NHCO-amide), 168.2 ($COOCH_3$), 169.5, 169.9, 169.9, 170.6, 170.7, 170.7 (8C, $COCH_3$), FAB-MS (mNBA) m/z $[M+H]^+$ 1259.0.

Methyl 2-deoxy-2-{methyl 2-deoxy-2-[methyl 2-deoxy-2-(methyl 2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside uronamido)-α-D-glucopyranoside uronamido]-α-D-glucopyranoside uronamido}-α-D-glucopyranoside uronic acid (69)

Composition **68** (0.13 g) was deacetylated to obtain 0.074 g (80%) of **69**, ^{1}H-NMR (DMSO-d_6+D_2O) δ 3.40, 3.41, 3.42 (3s, 12H, OCH_3), 3.65 (s, 3H, $COOCH_3$), 4.18-4.22 (4dd, 4H, H-5, 5',5",5"'), 4.70- 4.74 (4d, 4H, H-1,1',1",1"'), 7.51 (s, 4H, *Ph*), ^{13}C-NMR (DMSO-d_6+D_2O) δ 53.84, 53.85, 55.55, 55.56, 55.59, 55.93 (4x OCH_3, $COOCH_3$, C-2,2',2",2"'), 62.43 (OCH_2), 98.58, 98.72, 99.05 (4C, C-1,1',1",1"').

Structures of (2→6) linked homooligomer saccharopeptides



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62	n = 0	R_1 = Z	R_2 = H	R_3 = Me
63	n = 0	R_1 = Z	R_2 = H	R_3 = H
64	n = 0	R_1 = H	R_2 = H	R_3 = H
65	n = 0	R_1 = H	R_2 = Ac	R_3 = Me
66	n = 1	R_1= Z	R_2 = Ac	R_3 = Me
67	n = 1	R_1= Z	R_2 = H	R_3 = Me
68	n = 2	R_1 = Z	R_2 = Ac	R_3 = Me
69	n = 2	R_1 = Z	R_2 = H	R_3 = Me

Example 9

Synthesis of saccharopeptides - Heterooligomers having 1-amino-glucuronic acid C-terminal unit

N-(Methyl 2,3,4-tri-O-acetyl-β-D-glucopyran-1-osyl uronate)-(methyl 3,4-di-O-acetyl-2-benzyloxycarbonyl-amino-2-deoxy-α-D-glucopyranoside uronamide) (70)

Compositions 2 (0.70 g) and 10 (0.85 g) were dissolved in dichloromethane (10 mL), HOBT (0.40 g) and

DIC (0.47 mL) were added, and the reaction mixture was stirred overnight at room tenperature. The reaction was worked up as previously described, and the crude product was purified by column chromatography to get 1.20 g (81%) of **70**, ^{1}H-NMR ($CDCl_3$) δ 1.88, 2.01, 2.03, 2.05, 2.16 (5s, 20H, $COCH_3$), 3.41 (s, 3H, OCH_3), 3.72 (s, 3H, $COOCH_3$), 4.03 (ddd, 1H, H-2), 4.12 (d, 1H, H-5), 4.13 (d, 1H, H-5), 4.83 (d, 1H, H-1 $J_{1,2}$=3.5 Hz), 4.87 (t, 1H, H-4), 4.97 (t,, 1H, H-3'), 5.04 (d, 1H, OCH_2), 5.13 (t, 1H, H-4), 5.14 (d, 1H, OCH_2), 5.18 (t, 1H, H-1' $J_{1',2'}$=10.0 Hz), 5.24 (d, 1H, *N'H*), 5.40 (t, 1H, H-2'), 7.29 (d, 1H, *NH*), ^{13}C-NMR ($CDCl_3$) δ 20.43, 20.53, 20.56, 20.61 ($COCH_3$), 52.90 (OCH_3), 53.61 (C-2), 56.32 ($COOCH_3$), 67.02 (C-4), 68.58 (C-5), 69.26 (C-4'), 69.63 (OCH_2), 69.97 (C-3'), 70.18 (C-3), 71.65 (C-2'), 73.95 (C-5), 77.62 (C-1'), 98.70 (C-1), 125.29, 128.11, 128.22, 128.53, 129.01 (*Ph*), 136.23, 137.01 (Cq *Ph*), 157.88 ($OCOCH_2Ph$), 167.11, 168.02, 169.51, 169.54, 169.63, 170.62, 171.27 ((5x CO CH_3,*CO*NH, $COOCH_3$)).

N-(Methyl β-D-glucopyran-1-osyl uronate)-(methyl 2-benzyloxycarbonyl-amino-2-deoxy-α-D-glucopyranoside uronamide) (71)

Composition **70** (0.11 g) was deacetylated in methanol (10 mL) with sodium methoxide to yield **71** (78 mg, 98%), ^{1}H-NMR (DMSO-d_6+D_2O) δ 3.28 (s, O*CH*$_3$), 3.66 (s, COO*CH*$_3$), 3.75 (2d, H-5,5'), 4.80 (2d, H-1 $J_{1,2}$ 9 Hz, H-1',$J_{1',2'}$ 3.6 Hz), 7.38 (s, *Ph*), ^{13}C-NMR (DMSO-d_6+D_2O) δ 52.26, 55.47, 55.54 (O*CH*$_3$, COO*CH*$_3$, C-2), 65.74 (O*CH*$_2$), 70.23, 71.77, 72.07, 72.53, 76.49, 77.28 (7C, C-3,4,5,2',3',4',5'), 80.09 (C-1'), 99.06 (C-1), 128.10, 128.16 128.70 (*Ph*), 137.26 (C$_q$ *Ph*), 156.50, 169.76, 169.98 (3C, COO*CH*$_3$, *CO*NH).

N-(β-D-Glucopyran-1-osyl uronic acid)-(methyl 2-benzyloxycarbonyl-amino-2-deoxy-α-D-glucopyranoside uronamide) (72)

Composition **71** (0.11 g) was dissolved in 1 M NaOH (5 mL) and kept at 0 °C for one minute. After working up, the solution was lyophilized, to afford 75 mg (97%) of **72**, ^{1}H-NMR (D_2O) δ 3.38 (s, O*CH*$_3$), 3.75 (2d, H-1 $J_{1,2}$ 9.5 Hz, H-1',$J_{1',2'}$ 3.3 Hz), 4.00, 4.12 (2d, H-5,5'), 5.10 (2d, 2H, O*CH*$_2$), 7.40 (s, *Ph*), ^{13}C-NMR (D_2O) δ 55.11, 55.93 (O*CH*$_3$, C-2), 67.33 (O*CH*$_2$), 71.17, 71.44, 71.52, 71.70, 72.11, 76.32, 76.55, (7C, C-3,4,5,2',3',4',5'), 79.23 (C-1'), 99.13 (C-1), 127.94, 128.65 129.05 (*Ph*), 138.34 (C$_q$ *Ph*), 172.56 (2C, *CO*NH).

N-(β-D-Glucopyran-1-osyl uronic acid)-(methyl 2-amino-2-deoxy-α-D-glucopyranoside uronamide) (73)

Composition 72 (0.11 g) was hydrogenated overnight in the presence of 10 % palladium on charcoal (50 mg) at atmospheric pressure. The catalyst was filtered off, and the filtrate was evaporated to give 80 mg (88%) 73, ^{1}H-NMR (D_2O) δ 3.41 (dd, 1H, H-2), 3.52 (s, 3H, OCH_3), 3.52-3.62 (m, 3H), 3.65 (t, 1H), 3.81 (s, 3H, $COOCH_3$), 3.91 (t, 1H), 4.18 (d, 2H, H-5,5'), 5.11 (2d, H-1 $J_{1,2}$ 10.5 Hz, H-1',$J_{1',2'}$ 3.0 Hz), ^{13}C-NMR (D_2O) δ 53.36, 53.77, 55.98 (OCH_3, $COOCH_3$, C-2), 69.62, 71.29, 71.39, 71.54, 71.63, 76.16, 76.31 (7C, C-3,4,5,2',3',4',5'), 79.38 (C-1'), 96.80 (C-1), 171.09, 171.97 (2C, *CO*NH).

N-(Methyl β-D-glucopyran-1-osyl uronate)-(methyl 2-amino-2-deoxy-α-D-glucopyranoside uronamide) (74)

Composition 71 (84 mg) was hydrogenolysed in a mixture of methanol-water (10 mL), in the presence of 10% palladium on activated carbon, at atmospheric presssure. After complete reaction the catalyst was filtered off, the filtrate was evaporated to give the free amine derivative (74) (54 mg), ^{1}H-NMR (DMSO+D_2O) δ 3.35, 3.48 (2s, OCH_3, $COOCH_3$), 3.81 (2d, H-5,5'), 4.78 (2d, H-1,1'), ^{13}C-NMR (DMSO+D_2O) δ 52.34, 55.58, 55.62 (C-2, OCH_3, $COOCH_3$), 70.21, 70.89, 71.98, 72.45, 76.38, 77.32 (C-3,4,5,2',3',4',5'), 80.86 (C-1'), 99.14 (C-1), 169.68, 169.72 (*CO*NH, $COOCH_3$).

N-(Methyl 2,3,4-tri-O-acetyl-β-D-glucopyran-1-osyl uronate)-N-(methyl 3,4-di-O-acetyl-2-amino-2-deoxy-α-D-glucopyranoside uronate)-(1-azido-1-deoxy-2,3,4-tri-O-benzoyl-β-D-glucopyran-1-osyl uronamide) (75)

Composition 70 (0.55 g) was hydrogenated in ethyl acetate (10 mL), in the presence of 10 % palladium on charcoal (100 mg) to furnish the free amine derivative of the disaccharopeptide. The catalyst was filtered off, the solvent was evaporated. The residue and compound 7 (0.40 g) were dissolved in THF (10 mL). HOBT (0.15 g) and DIC (0.12 mL) were added and the reaction mixture was stirred for three days. The reaction was worked up as previously described and purified by column chromatography to provide the protected trisaccharopeptide (75) (0.76 g, 90%), ^{1}H-NMR ($CDCl_3$) δ 1.97, 2.02, 2.05, 2.14 (4s, 15H, $COCH_3$), 3.47 (s, 3H, $COOCH_3$), 3.70 (s, 3H, OCH_3), 4.18 (d, 1H, H-5'), 4.19 (d, 1H, H-5"), 4.21 (ddd, 1H, H-2'), 4.35 (d, 1H, H-5), 4.85 (d, 1H, H-1' $J_{1',2'}$=3.3 Hz), 4.94 (t, 1H, H-4'), 5.04 (t, 1H, H-2"), 5.11 (d, 1H, H-1, $J_{1,2}$=8.8 Hz), 5.20 (t, 1H, H-4"), 5.30 (t, 1H, H-1", $J_{1",2"}$=9.4 Hz), 5.38 (t, 1H, H-3'), 5.46 (t, 1H, H-3"), 5.55 (t, 1H, H-2), 5.65 (t, 1H, H-4), 6.02 (t, 1H, H-3), 6.88 (d, 1H, *N'H*), 7.14-7.30 (m, 3H, *Ph*, and *N"H*), 7.32-7.43 (m, 6H, *Ph*), 7.46-7.53 (m, 2H, *Ph*), 7.83 (d, 2H, *Ph*), 7.95, 7.98 (m, 3H, *Ph*), ^{13}C-NMR ($CDCl_3$) δ 20.41, 20.52, 20.58 (5C, $COCH_3$), 52.04 (C-2'), 52.86 (OCH_3), 56.31 ($COOCH_3$), 68.52 (C-5"), 69.12 (C-4'), 69.59 (2C, C-2",4"), 69.97 (C-3'), 70.03 (C-4), 70.85 (C-2), 71.79 (C-3"), 71.94 (C-3), 73.94 (C-5'), 74.80 (C-5), 77.58 (C-1"), 87.99 (C-1), 97.77 (C-1'), 128.22, 128.40, 128.44, 128.50, 128.71, 129.02, 129.66, 129.84, 129.85,

(*Ph*), 133.47, 133.52, 133.70 (3Cq *Ph*), 164.93, 165.28, 165.51, 166.12, 167.15, 168.12, 169.56, 169.57, 170.89, 171.00 (5x CO*CH*$_3$, 3x *CO*Ph, 2x*CO*NH, COO*CH*$_3$).

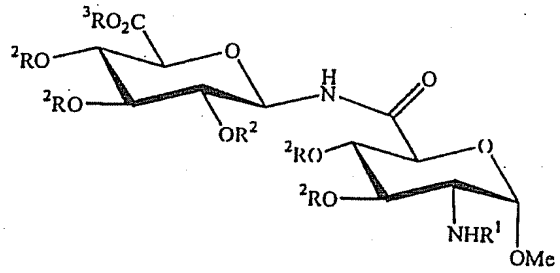
N-(Methyl β-D-glucopyran-1-osyl uronate)-N-(methyl 2-amino-2-deoxy-α-D-glu-copyranoside uronate)-(1-azido-1-deoxy-β-D-glucopyran-1-osyl uronamide) (76)

Composition **75** (0.56 g) was deacetylated in methanol (10 mL) with 1 M sodium methoxide to give 0.29 g (96 %) of **76**, ^{1}H-NMR (DMSO-d$_6$) δ 3.22 (t, 1H), 3.41 (s, O*CH*$_3$), 3.45 (3t, 3H), 3.59, 3.60 (2t, 2H), 3.62, 3.64, 3.66 (3t, 3H), 3.80 (s, COO*CH*$_3$), 3.87, 3.89, 4.00 (3d, 3H, H-5,5',5"), 4.66 (d, H-1" $J_{1",2"}$=8.7 Hz), 4.81 (d, 1H, H-1',$J_{1',2'}$ =3.4 Hz), 5.00 (d, 1H, H-1, $J_{1,2}$=8.4 Hz), ^{13}C-NMR (DMSO-d$_6$) δ 52.89, 53.64, 55.87 (O*CH*$_3$, COO*CH*$_3$, C-2'), 60.50, 70.33, 70.94, 71.53, 71.95, 72.30, 72.76, 75.67, 76.15, 76.83, 76.94, (C-2,3,4,5,2,3',4',5',2",3",4",5"), 79.23 (C-1"), 90.30 (C-1), 98.47 (C-1'), 169.29, 170.26, 171.15 (2x *CO*NH, *COO*CH$_3$).

N-(β-D-Glucopyran-1-osyl uronic acid)-N-(methyl 2-amino-2-deoxy-α-D-glucopyranoside uronate)-(1-azido-1-deoxy-β-D-glucopyran-1-osyl uronamide) (77)

Composition **76** (0.25 g) was treated with 1 M sodium hydroxide and worked up as previously described to provide **77** (0.24 g, 100 %), ^{13}C-NMR (D$_2$O) δ 53.51, 55.94 (O*CH*$_3$, C-2'), 70.54, 71.10, 71.36, 71.46, 71.71, 72.10, 72.60, 75.50, 76.29, 77.00 (11C, C-2,3,4,5,3',4',5',2",3",4",5"), 79.27 (C-1"), 90.46 (C-1), 98.51 (C-1'), 170.33, 172.42, 172.56 (2C, *CO*NH, COOH).

Structures of saccharopeptide heterooligomers having 1-amino-glucuronic acid C-terminal unit



70	R_1 = Z	R_2 = Ac	R_3 = Me
71	R_1 = Z	R_2 = H	R_3 = Me
72	R_1 = Z	R_2 = H	R_3 = H
73	R_1 = H	R_2 = H	R_3 = H
74	R_1 = H	R_2 = H	R_3 = Me
75	R_1= β-DGlcAAc	R_2 = Ac	R_3 = Me
76	R_1= β-DGlcA	R_2 = H	R_3 = Me
77	R_1 = β-DGlcA	R_2 = H	R_3 = H

β-DGlcAAc = 1-azido-1-deoxy-2,3,4-tri-O-acetyl-β-D-glucopyranuron-6-osyl

β-DGlcA = 1-azido-1-deoxy-β-D-glucopyranuron-6-osyl

Example 10

Synthesis of saccharopeptides - Heterooligomers having 2-amino-glucuronic acid C-terminal unit

Methyl {methyl 3,4-di-O-acetyl-2-deoxy-2-[(1-azido-1-deoxy 2,3,4-tri-O-benzoyl-β-D-glucopyran uronamido)-α-D-glucopyranoside]} uronate (78)

Compositions 12 (1.52 g) and 7 were dissolved in THF (10 mL) and DIC (2.34 mL) and HOBT (080 g) were added to the solution. The reaction mixture was stirred overnight and worked up as previously described. Column chromatography gave the protected disaccharopeptide (78), (3.27 g 80%), $[\alpha]_D$ +52.2° (*c* 1.38 chloroform), m.p. 130 °C (methanol), ^{1}H-NMR ($CDCl_3$) δ 2.00, 2.01 (2s, 20H, $COCH_3$), 3.61 (s, 3H, OCH_3), 3.76 (s, 3H, $COOCH_3$), 4.23 (ddd, 1H, H-2), 4.26 (d, 1H, H-5'), 4.33 (d, 1H, H-5), 4.99 (d, 1H, H-1' $J_{1,'2'}$=3.4 Hz), 5.04 (d, 1H, H-1 $J_{1,2}$=8.8 Hz), 5.21 (t, 1H, H-4'), 5.42 (t, 1H, H-3'), 5.52 (t, 1H, H-2), 5.58 (t, 1H, H-4), 5.98 (t, 1H, H-3), 6.80 (d, 1H, *NH*), 7.14-7.52 (12H, *Ph*), 7.82, 7.90, 7.96 (3d, 3H, *Ph*), ^{13}C-NMR ($CDCl_3$) δ 21.11, 21.21 ($COCH_3$), 52.49 (C-2), 53.45 (OCH_3), 56.74 ($COOCH_3$), 69.14 (C-5'), 69.77 (C-4'), 70.25 (C-3'), 70.59 C-4), 71.42 (C-2), 72.55 (C-3), 75.62 (C-5), 88.70 (C-1), 98.48 (C-1'), 125.88, 128.80, 128.97, 129.08, 129.26, 129.60, 130.30, 130.41, 130.49, 133.01, 134.06, 134.26 (*Ph*), 165.52, 165.90, 166.05, 166.48, 168.83, 170.03 (2x $COCH_3$, 3x CO*Ph*,*CO*NH, $COOCH_3$)), FAB MS $[M+H]^+$ 819.3, $[M+Na]^+$ 841.3.

Methyl [methyl 2-deoxy-2-(1-azido-1-deoxy-β-D-glucopyran uronamido)-α-D-glucopyranoside] uronate (79)

Composition 78 (0.21 g) was deacetylated as previously described to give 79 (100 mg, 95%), m.p. 190-191 °C (methanol), ^{1}H-NMR (D_2O) δ 3.32 (t, 1H), 3.41 (s, 3H, OCH_3), 3.55 (t, H), 3.58 (t, 1H), 3.66 (t, 1H), 3.79 (dd, 1H, H-2), 3.83 (s, 3H, $COOCH_3$), 4.01 (d, 1H, H-5), 4.06 (dd, 1H, H-2'), 4.25 (d, 1H, H-5), 4.82 (d, 1H, H-1 $J_{1,2}$=8.6 Hz), 4.88 (d, 1H, H-1' $J_{1',2'}$=3.3 Hz), ^{13}C-NMR (D_2O) δ 51.41, 51.56, 54.06 (OCH_3, $COOCH_3$, C-2'), 68.67, 69.12, 69.16, 69.93, 70.66, 73.56, 75.11 (C-2,3,4,5,3',4',5'), 88.58 (C-1), 96.64 (C-1'), 168.46, 169.87 ($CONH$, $COOCH_3$), FAB MS $[M+H]^+$ 423.3.

Methyl 2-deoxy-2-(1-azido-1-deoxy-β-D-glucopyran uronamido)-α-D-gluco-pyranoside uronic acid (80)

Composition 79 (0.10 g) was deprotected with sodium hydroxide to obtain the free acid (80), 95 mg (97%), $[\alpha]_D$ +24.6 (*c* 1.27, water), ^{1}H-NMR (D_2O) δ 3.30 (t, 1H), 3.40 (s, 3H, OCH_3), 3.54 (t, H), 3.60 (t, 1H), 3.63 (t, 1H), 3.79 (t, 1H, H-2), 4.01 (d, 1H, H-5), 4.04 (dd, 1H, H-2'), 4.15 (d, 1H, H-5), 4.81 (d, 1H, H-1 $J_{1,2}$=8.6 Hz), 4.85 (d, 1H, H-1' $J_{1',2'}$=3.5 Hz), ^{13}C-NMR (D_2O) δ 51.67, 54.04 (OCH_3, C-2'), 68.83, 68.22, 70.11, 70.71, 73.62, 75.15 (7C, C-2,3,4,5,3',4',5'), 88.62 (C-1), 96.55 (C-1'), 168.51, 171.76 ($CONH$, $COOH$), FAB MS $[M+H]^+$ 409.3, $[M-H]^-$ 407.1.

Methyl {methyl 3,4-di-O-acetyl-2-deoxy-2-[2,3,4-tri-O-benzoyl-1-(1-azido-1-deoxy 2,3,4-tri-O-benzoyl-β-D-glucopyran uronamido)-β-D-glucopyran uronamido]-α-D-glucopyranoside} uronate (81)

A solution of composition 78 (0.63 g) in EtOAc (20 mL) was hydrogenolyzed in the presence of 10% Pd-C (0.6 g) for 3.5 hours at room temperature. The mixture was filtered through Celite and the solids were washed with EtOAc. The combined filtrate and washings were concentrated to give the crude product as a white foam, which was used directly for the next step.

To a solution of the above product (0.61 g) and composition 7 (0.41 g) in anhydrous THF (10 mL) HOBT (0.52 g) was added at room temperature. After the mixture was stirred for 10 minutes, 1,3-diisopropylcarbodiimide (DIC, 0.61 mL) was added. The reaction mixture was stirred at room temperature for 9 days. During the reaction more 7 (200 mg) and coupling reagent (DIC, 0.30 mL) were added. After completion, the reaction mixture was diluted with dichloromethane, washed with water (3x50 mL), was dried with Na_2SO_4 and concentrated. Column chromatography (toluene-EtOAc, 3:1) of the residue on silica gel gave the title compound (81) (230mg, 25%) as a white foam: $[\alpha]_D$ +63.1 (*c* 1.60, chloroform); FAB Ms $[M-H]^-$ 1304.5; $[M-H]^-$ 1306.6; R_f 0.46 (toluene-acetone, 4:1).

Methyl {methyl 2-deoxy-2-[1-(1-azido-1-deoxy-β-D-glucopyran uronamido)-β-D-glucopyran uronamido]-α-D-glucopyranoside}uronate (82)

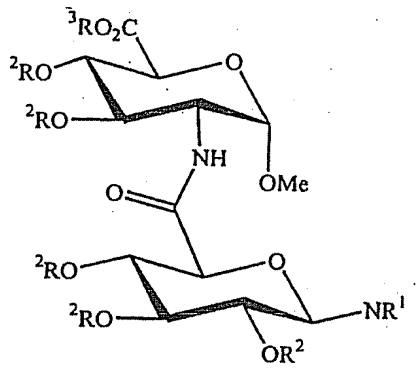
To a solution of 81 (230 mg) in MeOH (5 mL) was added 0.5 M NaOMe in MeOH (0.036 mL) at room temperature

to make the pH value of the reaction solution at 8-9. The reaction mixture was stirred at room temperature for 3 days. During the reaction more NaOMe (0:06 mL) was added. After completion, the reaction solution was neutralized with a cation-exchange resin [H^+], then the resin was filtered off and was washed with MeOH. The combined filtrate and washings were concentrated and the residue was purified by column chromatography ($CHCl_3$ - 10% aq. MeOH, 2:1) to give **82** (30mg, 30%) as a white solid:, $[\alpha]_D$ + 60.5 (*c* 1.0, water), R_f 0.35 ($CHCl_3$-10% aq. MeOH, 2:1); FAB MS: $[M+H]^+$ 598.3, $[M+Na]^+$ 620.3, $[M-H]^-$ 596.0.

Methyl 2-deoxy-2-[1-(1-azido-1-deoxy-β-D-glucopyran uronamido)-β-D-glucopyran uronamido]-α-D-glucopyranoside uronic acid (83)

A solution of composition **82** (15 mg) in 1 M aqueous NaOH was stirred at room temperature for 0.5 minute, then it was neutralized with a cation-exchange resin immediately. The resin was filtered off and washed with water. The combined water solution was lyophilized to give **83** (12mg, 82%) as a white powder, $[\alpha]_D$ + 87.3 (*c* 0.8, water), FAB MS: $[M-H]^-$ 582.0, [M-H+Na] 603.8.

Structures of saccharopeptide heterooligomers having 2-amino-glucuronic acid C-terminal unit



78	$R^1 = N_2$	R^2 = Ac	R_3 = Me
79	$R_1 = N_2$	R_2 = H	R_3 = Me
80	$R_1 = N_2$	R_2 = H	R_3 = H
81	R_1 = β-DGlcAAc	R_2 = Ac	R_3 = Me
82	R_1 = β-DGlcA	R_2 = H	R_3 = Me
83	R_1= β-DGlcA	R_2 = Hc	R_3 = H

β-DGlcAAc = 1-azido-1-deoxy-2,3,4-tri-O-acetyl-β-D-glucopyranuron-6-osyl

β-DGlcA = 1-azido-1-deoxy-β-D-glucopyranuron-6-osyl

Example 11

Synthesis of saccharopeptides - Heterooligomers having 2-amino-glucose C-terminal unit

Methyl 2-(1-azido-2,3,4-tri-O-benzoyl-1-deoxy-β-D-glucopyranuronamido)-α-D-glucopyranoside (84)

Piperidine (2 mL) was added to a solution of composition 15 (0.86 g, 2.07mmol) in DMF (15 mL) at room temperature. After 0.5 hour the reaction mixture was evaporated to dryness *in vacuo* and the crude methyl 2-amino-2-deoxy-α-D-glucopyranoside was used directly in the next step. A solution of methyl 2-amino-2-deoxy-α-D-glucopyranoside in DMF (15 mL), followed by DIC (0.59 mL) was added through a syringe to a mixture of composition 7 (1.0 g) and HOBT (0.28 g) in DMF (20 mL) which has been stirred under nitrogen at room temperature for 1.5 hour. After for 4 days, the reaction mixture was concentrated to dryness and the residue was purified by column chromatography (chloroform-methanol, 10:1) to give 84 (1.03 g, 77%) as a white foam; R_f 0.40 (chloroform-methanol, 10:1), $[\alpha]_D$ + 55.7 (*c* 1.15, chloroform); ESI-MS: $[M+Na]^+$ 729.3, $[M+H]^+$707.3.

^{1}H-NMR (CD_3OD): δ 7.30-8.00 (m, 15H, Ar), 6.01 (t, 1H, *J* = 9.6 Hz, H-3), 5.73 (t, 1H, *J*=9.8 Hz, H-4), 5.52 (t, 1H, *J*=9.1 Hz, H-2), 5.36 (d, 1H, *J*=8.9, H-1), 4.74 (d, 1H, J=3.4 Hz, H-1'), 4.56 (d, 1H, *J*=10.0Hz, H-5), 3.80-3.90 (m, 2H, H-2' and H-6'), 3.65-3.75 (m, 2H, H-3' and H-6'), 3.55-3.60 (m, 1H, H-5'), 3.46 (s, 3H, OMe), 3.34 (m, 1H, H-4').

^{13}C-NMR (CD_3OD): δ 167.08, 167.03, 166.71, 166.54 (C=O), 134.78-135.00 (Ar), 129.62-130.91 (Ar), 130.55, 130.21, 130.13 (Ar), 99.51, 89.58, 76.54, 74.25, 73.89, 72.68, 72.58, 72.42, 71.62, 62.81 (C-6), 55.82, 55.43.

Methyl 2-(1-azido-1-deoxy-β-D-glucopyran uronamido)-2-deoxy-α-D-gluco-pyranoside (85)

To a solution of 84 (120 mg) in MeOH (8 mL) 0.5M methanolic NaOMe (0.2 mL) was added. After stirring the reaction mixture at room temperature for 2 hours it was neutralized with AG W50-X8 (H^+) ion-exchange resin, then the resin was filtered off and was washed with methanol. The combined filtrate and washings were concentrated, the residue was dissolved in water and was extracted with ether. The water layer was lyophilized to give 85 (71 mg, 100%) as a white solid; $[\alpha]_D$ + 33.7 (*c* 0.86, water); R_f 0.53 (chloroform-10% aqueous methanol, 2:1); ESI-MS: $[M+Na]^+$ 417.6.

^{1}H-NMR (D_2O): δ 3.30-4.03 (m, 12H, sugar protons), 3.39 (s, 3H, OMe).

^{13}C-NMR (D_2O): δ 169.83 (C=O), 97.59, 89.95, 76.48, 74.92, 72.01, 71.37, 70.53 (2C) , 69.61, 60.21 (C-6), 54.89, 53.38.

Methyl 2-(1-azido-1-deoxy-β-D-glucopyran uronamido)-2-deoxy-α-D-glucopyranoside 6-O-sulfate (86)

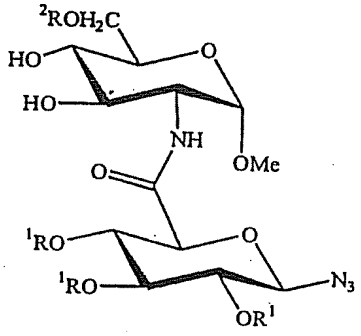
To a solution of composition 85 (50 mg) in pyridine (3 mL) sulfur trioxide pyridine complex (20 mg) was added and the mixture was stirred at room temperature. After 2 days additional sulfur trioxide pyridine (20 mg) was added and the mixture was stirred for 1 more day. Methanol was

added and the mixture was concentrated. The residue was purified on a C18 silicagel column by elution with water. The fraction containing the major product was stirred with AG W50-X8 [Na^+] resin, the resin was filtered and was washed with water. The filtrate was lyophilized to give 86 (30 mg, 50%) as a white solid; R_f 0.20 (chloroform-10% aqueous methanol, 2:1), $[\alpha]_D$ +27.2 (*c* 0.92, water), ESI-MS: $[M-Na]^-$ 473.3.

^{1}H-NMR (D_2O): δ 4.78-4.86 (2d, 2H, H-1,1′), 4.22-4.36 (2dd, 2H), 3.98-4.06 (m, 2H), 3.86-3.94 (m, 1H, H-5′), 3.74-3.82 (t, 1H, *J*=10.0 Hz), 3.51-3.65 (m, 3H), 3.40 (s, 3H, Ome), 3.30-3.36 (t, 1H).

^{13}C-NMR (D_2O): δ 169.79 (C=O), 97.60, 89.92 (C-1,1′), 76.46, 74.89, 72.00, 70.51, 70.46, 69.41, 69.28, 66.72 (C-6′), 54.98, 53.21.

Structures of saccharopeptide heterooligomers having 2-amino-glucose C-terminal unit



84	R^1 = Ac	R^2 = H
85	R_1 = H	R_2 = H
86	R_1 = H	R_2 = SO_3^-

Example 12

Synthesis of saccharopeptides - Saccharopeptides containing non-carbohydrate amino acids

Methyl 1-[2-(9-fluorenylmethoxycarbonyl)amino]-benzamido-2,3,4-tri-O-acetyl-1-deoxy-β-D-glucopyran uronate (87)

A solution of compound 2 (1.5 g) in anhydrous THF (30 mL), followed by DIC (1.74 mL), was added to a mixture of 2-fluorenyloxycarbonylamino-benzoic acid (2.0 g) and HOBT (0.83g) in THF (30 mL) which has been stirred under N_2 at room temperature for 1.5 hours. After stirring the reaction mixture at room temperature for 5 days, the solids were filtered and the filtrate was concentrated. Column chromatography of the residue gave 87 as a white solid, (1.15 g, 30%), R_f 0.32 (toluene-acetone, 8 :1); $[\alpha]_D$ -7.8 (c 0.64, chloroform-methanol, 10:1), ES-MS: $[M-H]^-$ 673.3, [M-Fmoc] 451.3. ^{1}H-NMR ($CDCl_3$+CD_3OD): δ 6.95-7.80 (m, 12H, Ar), 5.50 (d, 1H, J = 9.4 Hz, H-1), 5.48 (t, 1H, J =9.6 Hz, H-3), 5.20 (t, 1H, J =9.8 Hz, H-4), 5.14(t, 1H, J =9.4 Hz, H-2), 4.44 (d, 1H, J=1.5Hz, CH_aH_b-CH), 4.41 (s, 1H, CH_aH_b-CH), 4.36 (t, 1H, J =7.0 Hz, CH_2CH), 4.28 (d, 1H, J =10.1 Hz, H-5), 3.75 (s, 3H, OMe), 2.06-2.09 (3s, 9H, 3Ac).

^{13}C-NMR ($CDCl_3$): δ 171.58, 169.67, 169.55, 168.90, 167.05, 153,49, 143.85, 143.77, 141.27, 140.77, 133.91, 127.76, 127.15, 126.78, 125.30, 125.26, 122.24, 120.14, 119.99, 78.48, 74.08, 71.57, 70.38, 69.68, 67.41 (OCH2),53.04, 46.99, 20.74, 20.61, 20.49.

Methyl 1-[2-(9-fluorenylmethoxycarbonyl)amino]-benzamido-1-deoxy-β-D-glucopyran uronate (88)

To a solution of 87 (150 mg) in MeOH (10 mL) 0.5M methanolic NaOMe (0.05 mL) was added at room temperature to adjust the pH to ≈8. After the reaction mixture was stirred at room temperature for 2 hours the solution was neutralized with a cation-exchange resin [H^+ form], the resin was filtered and was washed with MeOH. The combined filtrate and washings were concentrated to give a white foam which was purified by column chromatography ($CHCl_3$-MeOH, 10:1) to give 88 (100 mg, 82%) as a white solid; R_f 0.42 ($CHCl_3$-MeOH, 10:1); $[\alpha]_D$ -23.0 (*c* 1.0, $CHCl_3$-MeOH, 10:1); ES-MS: $[M+H]^+$ 549.3, $[M+Na]^+$ 571.3. ^{1}H-NMR ($CDCl_3$+CD_3OD): δ 7.20-7.80 (m, 12H Ar), 5.26 (d, 1H, *J*=8.9 Hz, H-1), 4.40-4.45 (m, 2H, OCH_2CH), 4.30 (t, 1H, *J* =7.2 Hz, OCH_2CH), 4.10 (d, 1H, *J* = 9.3 Hz, H-5), 3.80 (s, 3H, OMe), 3.55-3.68 (m, 2H, H-4 and H-3), 3.50 (t, 1H, *J*=9.0 Hz, H-2). ^{13}C-NMR ($CDCl_3$+CD_3OD): δ 170.46, 170.06, 153.81, 143.87, 141.33, 139.83, 133.22, 127.84, 127.79, 127.24, 125.32, 122.12, 120.04, 119.98, 80.12, 76.84, 76.56, 71.93, 71.84, 67.43 (OCH_2CH), 52.78 (OCH_2CH), 47.07 (OMe).

Methyl 1-(2-amino)-benzamido-1-deoxy-β-D-glucopyran uronate (89)

To a solution of 88 (90 mg) in DMF (10 mL) piperidine (2 mL) was added at room temperature. After stirring the mixture for 0.5 hour, it was diluted with water (20 mL) and was extracted with hexanes to remove the by-product. The water layer was separated and lyophilized to give 89 as a white solid, (35 mg, 66%), $[\alpha]_D$ -180.0 (*c* 0.2, water); R_f 0.35 (chloroform-methanol, 5:1); ES-MS:

$[M+Na]^+$ 349.4, $[M+H]^+$ 327.4. ^{1}H-NMR (D_2O): δ 7.30-7.56 (m, 2H, Ar), 6.80-6.90 (m, 2H, Ar), 5.21 (d, 1H, *J*= 8.5 Hz, H-1), 4.20 (d, 1H, *J* = 9.2 Hz, H-5), 3.55-3.65 (m, 3H, H-2,-3,-4). ^{13}C-NMR (D_2O): δ 170.83 (C=O), 169.56(C=O), 132.06, 127.18, 116.68, 116.58, 78.36, 74,71, 74.63, 69.85, 69.79, 51.72 (CH_3).

1-(2-Amino)-benzamido-1-deoxy-β-D-glucopyran uronic acid (90)

A solution of 89 (20 mg) in 1M aqueous NaOH was stirred at room temperature for 0.5 minutes, then it was neutralized with an ion-exchange [H^+] resin immediately. The resin was filtered off and was washed with water. The combined water solution was lyophilized to give 90 (10mg, 52%) as a white solid; ^{1}H-NMR (D_2O): δ 7.40-7.60 (m, 2H, Ar), 6.90-7.00 (m, 2H, Ar), 5.18 (d, 1H, *J*=8.5 Hz, H-1), 3.89 (d, 1H, *J*= 9.1 Hz, H-5), 3.50-3.63 (m, 3H, H-2,-3,-4). ^{13}C-NMR (D_2O): δ 173.21, 173.17, 131.95, 127.26, 117.98, 117.35, 78.03, 76.51, 74.94, 70.31, 70.26.

N-(4-Methoxycarbonylphenyl) 1-azido-2,3,4-tri-O-benzoyl-1-deoxy-β-D-glucopran uronamide (91)

To a mixture of methyl 4-amino-benzoate (0.302 g) and compound 7 (1.062 g) in THF (20 mL), HOBT (2.702 g) and DIC (0.94 mL) were added. The mixture was stirred at room temperature. Additional amount of methyl 4-amino-benzoate (0.151 g), HOBT (1.351 g) and DIC (0.47 mL) were added after 3 days, and the mixture was stirred for 1 more day. It was evaporated, and the residue was subjected to column chromatography (toluene-ethyl acetate, 95:5) to give 91 (0.314 g, 23.7%).

^{13}C-NMR ($CDCl_3$+DMSO-d_6): δ 165.84, 164.87, 164.40, 164.28, 163.93 (C=Os), 87.55 (C-1), 75.50, 71.93, 70.22, 69.09, 51.37 (Me).

N-(4-Methoxycarbonyl-phenyl) 1-azido-1-deoxy-β-D-glucopyran uronamide (92)

Composition 91 (0.299) was debenzoylated with methanolic NaOMe, to yield 92 (0.107 g, 67.4%), $[\alpha]_D$ -55.4 (*c* 0.58, methanol).

^{1}H-NMR (CD_3OD): δ 4.67 (d, *J*=8.7 Hz, H-1), 4.00 (d, *J*=9.6 Hz, H-5), 3.88 (s, 3H, Me), 3.68 (t, 1H, H-4), 3.48 (t, 1H, H-3), 3.29 (t, 1H, H-2).

^{13}C-NMR (CD_3OD): δ 92.4 (C-1), 79.3, 77.6, 74.2, 72.6 (C-2,3,4,5), 169.1 and 168.1 (C=Os), 52.6 (Me).

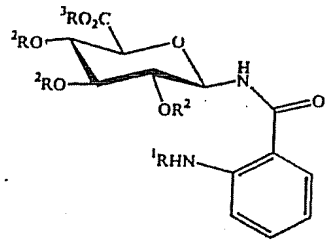
N-(4-carboxy-phenyl) 1-azido-1-deoxy-β-D-glucopyran uronamide (93)

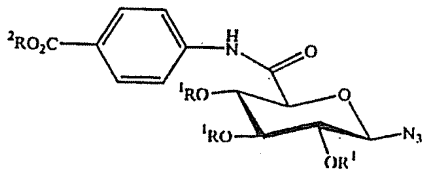
Composition 92 (0.090 g) was deesterified as described for 89 to give 93 (0.073 g, 84.7%).

^{1}H-NMR (H_2O): δ 7.92 (d, 2H, Ar), 7.55 (d, 2H, Ar), 4.82 (d, 1H, H-1), 4.04 (d, 1H, H-5), 3.63 (t, 1H, H-4), 3.54 (t, 1H, H-3), 3.31 (t, 1H, H-2).

^{13}C-NMR (H_2O): δ 171.5, 170.1 (C=O), 142.9, 132.6, 127.9, 122.5 (Ar), 92.1 (C-1), 79.0, 77.1, 74.3, 72.9 (C-2,3,4,5).

Structures of saccharopeptides containing non-carbohydrate amino acids





87	R^1 = Fmoc	R^2 = Ac	R^3 = Me
91	R^1 = Ac	R^2 = Me	
88	R^1 = Fmoc	R^2 = H	R^3 = Me
92	R^1 = H	R^2 = Me	
89	R^1 = H	R^2 = H	R^3 = Me
91	R^1 = H	R^2 = H	
90	R^1 = H	R^2 = H	R^3 = H

Example 13

N,N'-[bis(β-maltotriosyl)]-succinic diamide sulfate (98)

(a) A solution of maltotriose undecaacetate (1.93 g) in dichloromethane (10 ml) was treated with azidotrimethylsilane (0.4 ml) and tin (IV) chloride (0.18 ml), the reaction mixture was stirred overnight at room temperature, diluted with chloroform (50 ml) and extracted with saturated aqueous sodium bicarbonate, water, dried and evaporated. Column chromatography (toluene-ethyl acetate, 7:3 3:2) gave 1-azido-1-deoxy-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-(1-4)-O-(2,3,6-tri-O-acetyl- -D-glucopyranosyl)-(1-4)-2,3,6-tri-O-acetyl-α-D-glucopyranose (94, 1.82 g, 92%), n (N_3) 2123 cm^{-1}; ^1H-NMR

($CDCl_3$) δ 2.01, 2.015, 2.02, 2.03, 2.05, 2.06, 2.07, 2.11, 2.17, 2.20 (11s, $COCH_3$), 3.83 (ddd, 1H), 3.96 (m, 4H), 4.06 (dd, 1H), 4.19 (dd, 1H), 4,26 (dd, 1H), 4.33 (dd, 1H), 4.49 (2dd, 2H), 4.74 (2t, 2H), 4.77 (d 1H, H-1 $J_{1,2}$=8.7 Hz), 4.86 (dd, 1H), 5.08 (t, 1H), 5.28 (t, 1H), 5.29 (d, 1H, H-1a $J_{1,2}$=3.7 Hz), 5.36 (t, 1H), 5.43 (d, 1H, H-1a $J_{1,2}$=4.0 Hz), ^{13}C-NMR ($CDCl_3$) δ 20.56, 20.59, 20.68, 20.82, 20.91 (11C, $COCH_3$), 61.35, 62.25, 62.66 (C-6,6',6"), 67.85, 68.51, 69.05, 69.33, 70.07, 70.41, 71.51, 71.66, 72.42, 73.44, 74.15, 74.94 (C-2,3,4,5,2',3',4',5',2",3", 4",5"), 87.31 (C-1); 95.66, 95.91 (C-1',1"), 169.46, 169.54, 169.72, 169.87, 170.01, 170.36, 170.47, 170.55, 170.60 (11C, $COCH_3$), FAB-MS (mNBA) m/z $[M-H]^-$ 948.3, [M+mNBA] 1102.9.

A solution of **94** (1.31 g) in ethyl acetate (15 ml), was hydrogenated in the presence of Pd-C (0.3 g) at atmospheric pressure for one hour. Pd-C was filtered, and the solvent was evaporated to give deca-O-acetyl-β-maltotriosyl amine (**95**, 1.29 g, 100%); $[\alpha]_D$ +91.8° (*c* 1.01, chloroform), ^{1}H-NMR ($CDCl_3$) δ 1.98, 2.00, 2.01, 2.03, 2.06, 2.10, 2.16, 2.18 (11s, 33H, $COCH_3$), 3.71 (ddd, 1H), 3.94 (m, 4H), 4.00 (dd, 1H), 4.24 (m, 4H, 4.22, d, 1H, H-1, $J_{1,2}$=9.0 Hz), 4.44 (dd, 1H), 4.48 (dd, 1H), 4.46 (2t, 2H), 4.65 (t, 1H), 4.75 (dd, 1H), 4.85 (dd, 1H), 5.07 (t, 1H), 5.27 (d, 1H, H-1a $J_{1,2}$=4.7 Hz), 5.36 (t, 1H), 5.41 (d, 1H, H-1a $J_{1,2}$=3.8 Hz), ^{13}C-NMR ($CDCl_3$) δ 20.56, 20.63, 20.77, 20.88 (11C, $COCH_3$), 61.40, 62.37, 63.38 (C-6,6',6"), 67.92, 68.49, 68.92, 69.33, 70.10, 70.44, 71.66, 72.65, 72.95, 74.18, 75.57 (C-2,3,4,5,2',3',4',5',2",3", 4",5"), 84.35

(C-1); 95.65 (2C, C-1',1"), 169.40, 169.65, 169.77, 169.90, 170.29, 170.44, 170.53, 170.62 (11C, $COCH_3$)

To a solution of 95 (0.89 g) in dichloromethane (10 ml) at 0°C, pyridine (0.085 ml) followed by succinyl dichloride (0.05 ml) were added droppwise. The reaction mixture was stirred overnight at room temperature, diluted with chloroform (50 ml), washed with saturated aqueous $NaHCO_3$ and water. The organic layer was dried and evaporated, and the crude product was purified by column chromatography (toluene-acetone, 3:2) to yield N,N'-[bis(deca-O-acetyl-β-maltotriosyl)]-succinic diamide (96, 0.93 g, 45%); $[\alpha]_D$ +93.9° (c 1.28, chloroform), ^{1}H-NMR ($CDCl_3$) δ 2.01, 2.01, 2.03, 2.04, 2.06, 2.07, 2.10, 2.15, 2.17 (11s, 33H, $COCH_3$), 2.37, 2.55 (2d, 2H, $COCH_2$), 3.80 (ddd, 1H), 3.94 (m, 4H), 4.05 (dd, 1H), 4.16 (dd, 1H), 4,25 (dd, 1H), 4.29 (dd, 1H), 4.45 (2t, 2H), 4.74 (dd, 1H), 4.77 (dd, 1H, H-1 $J_{1,2}$=9.5 Hz), 4.85 (dd, 1H), 5.07 (t, 1H), 5.22 (t, 1H), 5.26 (d, 1H, H-1a $J_{1,2}$=4.1 Hz), 5.36 (dd, t, 2H), 5.39 (t, 1H), 5.41 (d, 1H, H-1a $J_{1,2}$=4.1 Hz), 6.37 (d, 1H, NHCO); ^{13}C-NMR ($CDCl_3$) δ 20.60, 20.69, 20.83, 20.90 (11C, $COCH_3$), 30.62 ($COCH_2$), 61.36, 62.21, 62.86 (C-6,6',6"), 67.88, 68.47, 69.06, 69.36, 70.08, 70.43, 71.19, 71.64, 72.44, 73.66, 73.93, 74.83 (C-2,3,4,5,2',3',4',5',2";3",4",5"), 77.31 (C-1); 95.63, 95.81 (C-1',1"), 169.48, 169.59, 169.63, 169.80, 170.36, 170.54, 170.57, 170.65, 170.73, 171.33, 171.76 (12C, $COCH_3$, $COCH_2$), FAB-MS (mNBA) m/z $[M+H]^+$ 1929.6, $[M-H]^-$ 1927.0.

A solution of 96 (0.49 g) in a mixture of methanol and water (2:1, 9 ml) was treated with methanolic sodium methoxide to adjust the pH to 8. The mixture was stirred

overnight at 0°C, and neutralized with AG 50 W-X8 (H^+) resin. The resin was filtered, the filtrate was evaporated and the residue was dried in vacuo to yield N,N'-[bis(β-maltotriosyl)]-succinic diamide (97, 0.25 g, 90%), ^{1}H-NMR (D_2O) δ 2.62 (dd, 2H, $COCH_2$), 3.42 (2dd, 2H), 3.58 (dd, 1H), 3.60 (dd, 1H), 3.66 (m, 5H), 3.72 (dd, 1H), 3.78 (2dd, 2H), 3.84 (m, 6H), 3.96 (t, 1H), 4.97 (d, 1H, H-1 $J_{1,2}$=9.2); 5.39 (d, 1H, H-1a $J_{1,2}$=4.1 Hz), 5.40 (d, 1H, H-1a $J_{1,2}$=4.3 Hz), ^{13}C-NMR (D_2O) δ 30.55 ($COCH_2$), 60.71 (3C, C-6,6',6"), 69.56, 71.43, 71.77, 71.91, 72.00, 72.95, 73.12, 73.56, 76.34, 76.70, 77.02, 77.11 (C-2,3,4,5,2',3',4',5',2",3",4",5"), 79.37 (C-1), 99.74, 100.03 (C-1',1"), 176.21 ($COCH_2$); FAB-MS (mNBA) m/z [M-H]$^-$ 1087.1.

A solution of 97 (0.19 g) in DMF (5 ml) 2,6-di-t-butyl-4-methylpyridine (1.51 g) was treated with sulfur trioxide pyridine complex (1.16 g) and the mixture was stirred at room temperature for three days. The reaction mixture was cooled to 0°C and the pH was adjusted to 8 using saturated aqueous $NaHCO_3$, and the solvent was evaporated. The residue was desalted on a Biogel P-2 column using 0.5 M ammonium bicarbonate as eluant. The carbohydrate containing fractions were pooled and lyophilized. The resulting product was passed through an SP Sephadex C-25 (Na^+) column with water to yield the sodium salt of N,N'-[bis(β-maltotriosyl)]-succinic diamide sulfate (98, 0.52 g, 92%).

(b) NH_4HCO_3 (6.32 g) was added to a solution of maltotriose (2.02 g) in water, and the mixture was stirred at room temperature. Additional NH_4HCO_3 (6.32 g) was added

after 3 days. TLC (isopropyl alcohol-acetone-water, 4:2:1) indicated complete conversion after one week, and the mixture was lyophilized. The resulting maltotriosyl amine (2.02 g) was dissolved in water (10 ml), aqueous $NaHCO_3$ was added to pH 9, the mixture was cooled to $0^{\circ}C$, and succinyl dichloride (0.16 ml) was added dropwise. The mixture was evaporated and the residue was purified by column chromatography (chloroform-90% aqueous methanol, 1:1) to yield **97**. Sulfation of **97**, as described above yielded **98**.

Example 14

N,N'-[bis(β-maltosyl)]-succinic diamide sulfate (103)

A solution of maltose octaacetate (6.78 g) in dichloromethane (50 ml) was treated with azidotrimethylsilane (1.8 ml) and tin (IV) chloride (1.0 ml), and the mixture was stirred overnight at room temperature. The reaction mixture was worked up as described previously and the crude product was recrystallized from ethanol to give 1-azido-1-deoxy-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-α-D-glucopyranose (99, 6.28 g, 95%).

A solution of 99 (0.67 g) in ethyl acetate (15 ml), was hydrogenated in the presence of Pd-C (0.3 g) at atmospheric pressure for one hour. Pd-C was filtered, and the solvent was evaporated to give 1-amino-1-deoxy-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranose (100, 0.64 g, 100%).

A solution of 100 (0.64 g) and succinic acid (0.06 g) in THF (8 ml) was treated with DIC (0.24 ml) in the presence of HOBT (50 mg) and the mixture was stirred for three days at room temperature (TLC - toluene-acetone, 3:2). The solvent was evaporated and the residue was purified by column chromatography (toluene-acetone, 4:1 7:3) to give N,N'-[bis(hepta-O-acetyl-β-maltosyl)]-succinic diamide (101, 0.56 g, 84%).

A solution of 101 (0.34 g) in methanol (20 ml) was treated with methanolic sodium methoxide (pH 9) overnight at 0°C . The product crystallized spontaneously from the solution, was filtered and washed with cold methanol to

furnish N,N'-[bis(β-maltosyl)]-succinic diamide (102, 0.18 g, 95%).

Sulfation of 102, as described above yielded N-[bis(β-maltosyl)]-succinic diamide sulfate (103).

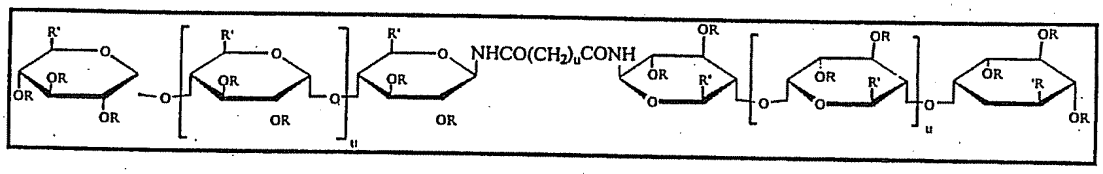
Example 15

N,N'-[bis(β-maltosyl)]-adipic diamide sulfate (106)

A solution of hepta-O-acetyl-β-D-maltosyl amine (100) (0.64 g) and adipic acid (0.07 g) in THF (6 ml) was treated with DIC (0.25 ml) and HOBT (50 mg) as described above, to yield N,N'-[bis(hepta-O-acetyl-β-maltosyl)]-adipic diamide. The crude product was purified by column chromatography (toluene-acetone, 4:1) to give final product 104 (0.24 g, 34%), $[\alpha]_D$ 55.60 (*c*, 1.00, chloroform).

A solution of 104 (0.24 g) was deacetylated in methanol (10 ml) as described above to give N,N'-[bis(β-maltosyl)]-adipic diamide (105, 0.12 g, 90%). Sulfation of 105 was carried out as described above to give N,N'-[bis(β-maltosyl)]-adipic diamide sulfate 106.

The structures of compositions 98, 103 and 106 are shown below



Composition 98, u = 1, t=2; Composition 103, u=0, t=2; Composition 106, u=0, t=4;

For all three compositions R= $-SO_3^-$, R'=$-CH_2OSO_3^-$

Example 16

N,N'-[bis(β-D-cellobiosyl)]-succinic diamide sulfate (39)

A solution of cellobiose octaacetate (6.78 g) in dichloromethane (50 ml) was treated with azidotrimethylsilane (1.8 ml) and tin (IV) chloride (1.0 ml), and the mixture was stirred overnight at room temperature. The reaction mixture was worked up as described previously and the crude product was recrystallized from ethanol to give 1-azido-1-deoxy-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranose (107, 6.48 g, 98%).

A solution of 107 (0.67 g) in ethyl acetate (15 ml) was hydrogenated in the presence of Pd-C (0.3 g) at atmospheric pressure for one hour. Pd-C was filtered, and the solvent was evaporated to give 1-amino-1-deoxy-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranose (108, 0.64 g, 100%).

A solution of 108 (0.64 g) and succinic acid (0.06 g) in THF (8 ml) was reacted with DIC (0.24 ml) in the presence of HOBT (50 mg) as described above to give N,N'-[bis (hepta-O-acetyl-β-cellobiosyl)]-succinic diamide (109, 0.58 g, 86%), $[\alpha]_D$ -7.1° (*c*, 1.00, chloroform), ^{1}H-NMR ($CDCl_3$) δ 1.98, 2.01, 2.02, 2.03, 2.06, 2.09, 2.11 (7s, 21H, $COCH_3$), 2.42 (m, 2H, $COCH_2$), 3.65 (ddd, 1H, H-5'), 3.67 (ddd, 1H, H-5), 3.74 (t, 1H, H-4, $J_{4,5}$=9.0 Hz), 4.01

(dd, 1H, H-6'a, $J_{5',6'a}$=1.9 Hz), 4,09 (dd, 1H, H-6b, $J_{5,6b}$=4.2 Hz), 4.36 (dd, 1H, H-6'b, $J_{5',6'b}$=3.8 Hz), 4.43 (dd, 1H, H-6a, nd), 4.49 (d, 1H, H-1', $J_{1',2'}$=8.0 Hz), 4.82 (t, 1H, H-2 $J_{2,3}$=9.6 Hz), 4.91 (t, 1H, H-2', $J_{2',3'}$=8.4 Hz), 5.05 (t, 1H, H-3', $J_{3',4'}$=9.6 Hz), 5.11 (t, 1H, H-4', $J_{4',5'}$=9.3 Hz), 5.13 (deuterated 5.18, d, 1H, H-1 $J_{1,2}$=9.3 Hz), 5.23 (t, 1H, H-3, $J_{3,4}$=9.1 Hz), 6.51 (d, 1H, *NH*CO, $J_{1,NH}$=9.3 Hz); ^{13}C-NMR ($CDCl_3$) δ 20.51, 20.64, 20.87 (7C, $COCH_3$), 30.55 ($COCH_2$), 61.54 (C-6'), 61.79 (C-6), 67.78 (C-3'), 70.56 (C-2), 71.50 (C-2'), 71.82 (C-5), 72.58 (C-3), 72.90 (C-4'), 74.62 (C-5'), 76.14 (C-4), 77.81 (C-1), 100.66 (C-1'), 169.07, 169.31, 169.50, 170.20, 170.29, 170.49, 170.88 (7C, $COCH_3$), 172.37 ($COCH_2$), FAB-MS (mNBA) m/z $[M+H]^+$ 1353.4, $[M-H]^-$ 1351.0.

A solution of **109** (0.19 g) in methanol (10 ml) was deacetylated as described above, to yield the crystalline N,N'-[bis(β-cellobiosyl)]-succinic diamide (**110**, 0.1 g, 98%), $[\alpha]_D$ -18.4° (*c* 0.98, water), ^{1}H-NMR (D_2O) δ 2.65 (dd, 2H, $COCH_2$), 3.31 (t, 1H), 3.45 (m,4H), 3.68 (m, 3H), 3.76 (dd, 1H), 3.81 (dd, 1H), 3.92 (dd, 2H), 4.51 (d, 1H, H-1 $J_{1',2'}$=8.0 Hz); 4.99 (d, 1H, H-1 $J_{1'2}$=9.3 Hz Hz), ^{13}C-NMR (D_2O) δ 30.23 ($COCH_2$), 60.04, 60.81 (C-6,6'), 69.68, 71.80, 73.37, 75.19, 75.69, 76.22, 76.56, 78.29 (C-2,3,4,5,2',3',4',5'), 79.33 (C-1), 102.74 (C-1'), 176.23 ($COCH_2$); FAB-MS (mNBA) m/z $[M-H]^-$ 763.5.

Sulfation of 110 (0.05 g) as described above yielded N, N'-[bis(β-D-cellobiosyl)]-succinic diamide sulfate (111, 0.25 g, 89%).

Example 17

N,N'-[bis(β-D-cellobiosyl)]-3-hydroxy-3-methylglutaric diamide sulfate (114)

Compound 108 (0.64 g) and 3-hydroxy-3-methylglutaric acid (0.08 g) was reacted in 1,4-dioxane (8 ml) with DIC (0.24 ml) in the presence of HOBT (50 mg) as described above to give N,N'-[bis(hepta-O-acetyl-β-cellobiosyl)]-3-hydroxy-3-methylglutaric diamide (112, 0.53 g, 76%), $[\alpha]_D$ -11.7° (*c* 1.06, chloroform), ^{1}H-NMR ($CDCl_3$) δ 1.26 (s, 3H, CH_3), 1.99, 2.01, 2.02, 2.05, 2.10 (14s, 51H, $COCH_3$), 2.35 (m, 4H, $COCH_2$), 3.71 (m, 4H), 3.86 (t, 2H), 4.03 (dd, 2H), 4.23 (ddd, 1H H-5), 4.40 (2t, 4H), 4.56 (d, 2H, H-1', $J_{1',2'}$=8.0 Hz), 4.87 (dd, 1H), 4.93 (t, dd, 4H), 5.09 (t, 2H, H-1 $J_{1,2}$=9.3 Hz), 5.18 (m, 6H), 7.37, 7.41 (2d, 2H, *NH*CO), ^{13}C-NMR ($CDCl_3$) δ 20.55, 20.60, 20.61, 20.67, 20.87 (14C, $COCH_3$), 27.27 (CH_3), 45.97, 46.97 ($COCH_2$), 61.56, 61.63, 61.69 (4C, C-6,6'), 70.40 ($C(CH_3)OH$), 67.78, 70.36, 70.42, 71.54, 71.89, 72.81, 73.05, 73.17, 74.94, 75.02, 76.18 (16C, C-2,3,4,5,2',3', 4',5'), 77.71 (2x C-1), 100.67 (2x C-1'), 169.00, 169.03, 169.29, 169.59, 169.64, 170.26, 170.49, 170.51, 170.57, 170.59, 170.66 (14C, $COCH_3$), 171.82, 172.60 ($COCH_2$), FAB-MS (mNBA) m/z $[M+H]^+$ 1396.8, $[M-H]^-$ 1395.0.

A solution of 112 (0.34 g) in methanol (10 ml) was deacetylated as described above, yielded the crystalline

N,N'-[bis(β-cellobiosyl)]-3-hydroxy-3-methylglutaric diamide (113, 0.19 g, 98%), $[\alpha]_D$ -4.8° (*c* 1.03, water), ^{1}H-NMR (D_2O) δ 1.38 (s, 3H, CH_3), 2.63 (m, 4H, $COCH_2$), 3.32 (t, 2H), 3.45 (m, 8H), 3.67 (m, 8H), 3.74 (t, 1H), 3.83 (dd, 3H), 3.93 (dd, 4H), 4.51 (d, 2H, H-1 $J_{1,2}$=7.7 Hz), 5.00 (d, 2H, H-1 $J_{1,2}$=9.3 Hz Hz), ^{13}C-NMR (D_2O) δ 26.60 (CH_3), 47.01, 47.18 ($COCH_2$), 60.06, 60.84 (4C, C-6,6'), 70.90 (*C* (CH_3)OH), 69.69, 71.78, 73.38, 75.27, 75.71, 76.24, 76.64, 78.31 (16C, C-2,3,4,5,2', 3',4',5'), 79.26 (2x C-1), 102.76 (2x C-1'), 174.41 (2C, $COCH_2$).

Sulfation of 113 (0.16 g) as described above yielded N,N'-[bis(β-D-cellobiosyl)]-3-hydroxy-3-methylglutaric diamide sulfate (114, 0.41 g, 91%).

Example 18

Sulfation of Tetrasaccharopeptide (60)

A solution of composition 60 (100 mg) in a mixture of methanol-water (1:1, 10 ml) with 1M NaOH (0.3 ml) is stirred overnight at room temperature. The mixture is neutralized with AG 50X H+ from ion-exchange resin. The resin filtered, and the solvent is evaporated to furnish the free hydroxyl containing uronic acid derivative.

A solution of uronic acid derivative in DMF (5 ml) with sulfur trioxide pyridine complex (100 mg) is stirred for 3 days at room temperature. The mixture is neutralized with $NaHCO_3$ to a pH of about 8, evaporated, desalted on a biogel P-2 column using ammonium bicarbonate (0.5M), the ammonium salt is converted to the sodium salt by passing through a SP-Sephadex ion-exchange column which yields the sulfated N-protected tetrasaccharopeptide 115.

Example 19

Solid Phase Synthesis

Composition 11 (427 mg) is deacetylated and hydrogenated as described above to give the free amino derivative (200 mg). The free amine is treated with BOC-ON {2-tert-butoxycarbonyloxy-imino)-2-phenylacetonitrile, 390 mg} in dioxane (10 ml) and triethyl amine (TEA , 0.2 ml), the solvent is evaporated, and the residue is dried, redissolved in pyridine (10 ml), cooled to 0°C and acetic

anhydride (0.5 ml) is added dropwise into the solution. The reaction mixture is stirred overnight, poured into ice-water, extracted with chloroform, evaporated and the residue is recrystallized from ethanol to yield Boc-sugar amino acid methyl ester (380 mg). Selective deesterification of the Boc-derivative, as earlier described, yields methyl 3,4-di-O-acetyl-2-*t*-butoxycarbonylamino-2-deoxy-α-D-glucopyranosyl uronic acid (116). Composition 116 is linked to the Merrifield resin as described by Merrifield. (Merrifield, R. B., Biochemistry, (1964) 3:1385; Erickson, B. W. and Merrifield, R. B., The Proteins, Neurath, H. and Hill, R. L. (eds), Vol.2, 3rd edn, Academic Press, New York, 255-527 (1979); Barany, G. and Merrifield, R. B., The Peptides, Gross, E. and Meienhofer, J. (eds), Vol.2, Academic Press, New York, 3-285 (1979).)

The Boc group is removed for further elongation, by treating the N-protected, resin linked sugar with trifluoroacetic acid (0.5 ml) in dichloromethane (20 ml) to give the free amino derivative (117) which was treated with TEA (0.2 ml) in dichloromethane (20 ml). Coupling 116 and 117 using the previously described method yields the resin linked protected disaccharopeptide. The protecting groups are removed as described above, and the saccharopeptide is detached from the resin treating it with hydrogen fluoride as described by Merrifield. (Merrifield, R. B., Biochemistry, (1964) 3:1385; Erickson, B. W. and Merrifield, R. B., The Proteins, Neurath, H. and Hill, R. L. (eds), Vol.2, 3rd edn, Academic Press, New York, 255-527 (1979); Barany, G. and Merrifield, R. B.,

The Peptides, Gross, E. and Meienhofer, J. (eds), Vol.2, Academic Press, New York, 3-285 (1979).

Example 20

Combinatorial Synthesis

Methyl polyethylene glycolyl (MeOPEGyl) (1-Azido-1-deoxy-2,3,4-tri-O-benzoyl-β-D-glucopyranose) uronate (118)

To a solution of 7 (1.06 g) in dichloromethane (10 mL) N,N-dimethylformamide (0.31 mL) was added and the reaction mixture was cooled to 0 °C. Oxalyl chloride (0.38 mL) was added dropwise to the solution and the mixture was stirred for 20 min.

Polyethylene glycol monomethyl ether (7.50 g) was dissolved in anhydrous dichloromethane (30 mL). Pyridine (1.21 mL) was added into the solution, followed by the previously prepared acyl chloride. After 30 min the reaction mixture was poured into ice-water, and diluted with dichloromethane (500 mL). The organic layer was separated, then subsequently extracted with water, sat. sodium hydrogen carbonate, water, was dried and evaporated. The residue was recrystallized from hot ethanol to give 118, 8.00 g (96%), IR (n)N_3 2119 cm^{-1}, ^{1}H-NMR ($CDCl_3$): δ 3.38 (s, OPEGO*CH*$_3$), 3.50 (PEG-*methylenes*), 4.50 (d, 1H, H-5), 5.04 (d, 1H, H-1 $J_{1,2}$=9.3 Hz), 5.45 (t, 1H), 5.65 (t, 1H), 5.86 (t, 1H), 7.28-7.58 (*Ph*), 7.84, 7.96 (m, *Ph*), ^{13}C-NMR ($CDCl_3$): δ 65.23, 68.32, 71.93, 74.34 (C-2,3,4,5), 88.22 (C-1), 128.35, 128.46, 129.72, 129.80, 129.84, (*Ph*), 133.44, 133.57 (q, *Ph*), 164.56, 164.57, 165.40, 166.15 (*CO*Ph, *CO*OPEG).

MeOPEGyl [methyl 3,4-di-O-acetyl-2-deoxy-2-(9-fluorenylmethoxycarbonyl-amino)-α-D-glucopyranosidel uronate (119)

To a solution of polyethylene glycol monomethyl ether (MeOPEGOH) (average m.w. 5,000) (7.50 g) in a mixture of anhydrous tetrahydrofuran (30 mL) and anhydrous dichloromethane (10 mL) a solution of 17 (1.03 g) and HOAT (0.54 g) was added followed by 1,3-diisopropyl-carbodiimide (DIC) (0.77 mL). The reaction mixture was stirred for three days at room temperature, then half of the solvent was evaporated. The MeOPEG derivative was precipitated with *tert.*-butyl-methyl ether. The white solid was filtered off and was redissolved in hot ethanol (50 mL), cooled to 5 °C until precipitation was completed. The product was separated by filtration, washed with *t*-butyl-methyl ether and dried in vacuo to yield 7.84 g (95%) of 119, ^{1}H-NMR ($CDCl_3$): δ 1.94, 2.04 (2s, 6H, $COCH_3$), 3.32 ($OPEGOCH_3$), 3.42 (OCH_3), 3.66 (PEG-*methylenes*), 4.08 (t, 1H), 4.20 (d, 1H, H-5), 4.28 (m, 2H), 4.42 (t, 1H), 4.84 (d, 1H, H-1), 5.16 (m, 1H), 5.28 (t, 1H), 7.32, 7.40, 7.55, 7.76 (9H, Ph, NH): ^{13}C-NMR ($CDCl_3$): δ 20.50, 20.63 ($COCH_3$), 47.01 (*CH*Fmoc), 53.43, 56.02 (OCH_3, C-2), 65.00, 68.62, 69.22 (C-3,4,5), 67.00 (CH_2Fmoc), 98.68 (C-1), 119.96, 124.94, 125.00, 127.05, 127.72 (*Ph*), 141.22, 143.57, 143.67 (q, *Ph*), 155.65 (*CO*Fmoc), 167.77, 169.36, ($COCH_3$), 170.73 (*CO*OPEGOMe).

MeOPEGyl [methyl 3,4-di-O-acetyl-2-deoxy-2-(1-azido-1-deoxy-2,3,4-tri-O-benzoyl-β-D-glucopyran uronamido)-α-D-glucopyranosidel uronate (121)

The Fmoc protecting group of 119 (1.10 g) was removed in N,N-dimethylformamide (10 mL) containing 20% piperidine.

The product was precipitated with *t.*-butyl-methyl ether, was filtered off and recrystallized from ethanol to give the free amine (120) 1.0 g (95%) , which was directly used in the next step. Composition 120 was dissolved in mixture of anhydrous tetrahydrofuran (10 mL) and dichloromethane (5 mL) and a solution of 7 (0.11 g) and HOAT (0.03 g) in THF (5 mL) was added followed by DIC (0.05 mL). The reaction mixture was stirred overnight at room temperature, and worked up as described previously, to obtain 121 (1.05 g, 91%); ^{1}H-NMR ($CDCl_3$): δ 1.98, 2.03 (2s, $COCH_3$), 4.23 (m, 2H), 4.34 (d, 1H), 4.98 (d, 1H, H-1' $J_{1',2'}$=3.6 Hz), 5.09 (t, 1H, H-1 $J_{1,2}$=8.8 Hz), 5.18 (t, 1H), 5.39 (t, 1H), 5.51 (m, 2H), 5.92 (t, 1H), 6.78 (d, *NH* *J*=8.2 Hz), 7.40, 7.82, 7.92 (m, 16H, *Ph*, *NH*); ^{13}C-NMR ($CDCl_3$): δ 20.50, 20.55 ($COCH_3$), 51.83, 56.12 (C-2', OCH_3), 64.95, 68.59, 69.02, 69.66, 69.95, 71.85, 74.93 (C-2,3,4,5,3',4',5'), 87.97 (C-1), 97.76 (C-1'), 128.34, 128.45, 129.62, 129.75, 129.82 (*Ph*), 133.37, 133.42, 133.62 (q, *Ph*), 164.80, 165.20, 165.33 (*CO*Ph), 165.78 (*CO*NH), 167.83, 169.35 ($COCH_3$), 170.96 (*CO*OPEGOMe).

MeOPEGyl [methyl 3,4-di-O-acetyl-2-deoxy-2-(3-fluorenylmethoxycarbonyl-amino-benzamido)-α-D-glucopyranoside] uronate (122)

Compound 119 (3.35 g) was deprotected as described earlier to give 120 (2.98 g, 94 %), which was coupled in a mixture of THF (25 mL) and dichloromethane (15 mL) with 3-fluorenylmethoxycarbonylamino-benzoic acid (0.43 g) in the presence of HOAT (0.16 g) and DIC (0.2 mL). After work-up as previously described, 2.93 g (93%) of 122 title product was isolated. ^{1}H-NMR ($CDCl_3$): δ 1.98, 2.06 (2s, 6H, $COCH_3$), 4.26 (m, 2H, CH_2Fmoc), 4.34 (d, 1H, H-5), 4.56 (m, 1H, H-2),

4.63 (d, 1H), 4.84 (d, 1H, H-1 $J_{1,2}$=2.5 Hz), 5.24 (t, 1H), 5.38 (t, 1H), 6.45 (d, 1H, *NH* J=9.5 Hz), 7.38, 7.66, 7.78 (m, 13H, *Ph*, *NH*); ^{13}C-NMR ($CDCl_3$): δ 20.54, 20.64 ($COCH_3$), 47.10 (*CH*Fmoc), 52.00, 55.99 (OCH_3, C-2), 64.99, 68.54, 69.18 (C-3,4,5), 66.00 (CH_2Fmoc), 98.45 (C-1), 119.89, 121.25, 124.82, 127.10, 127.68, 129.11 (*Ph*), 134.24, 139.00, 141.32, 143.76 (q, *Ph*), 153.48 (*CO*Fmoc), 166.88 (*CO*NH), 167.38, 169.35, ($COCH_3$), 171.19 (*CO*OPEGOMe).

Synthesis of a 5 component saccharo-tripeptide library

Composition 122 (2.70 g) was deprotected as described earlier to afford the free amino derivative (123). This was coupled in a mixture of THF (25 mL) and dichloromethane (10 mL) with the following carboxylic acids: 7 (51 mg), 19 (61 mg), 40 (61 mg), 3-fluorenylmethoxycarbonylamino-benzoic acid (34 mg) and N-fluorenylmethoxycarbonyl-β-alanine (29 mg) in the presence of HOAT (0.33 g) and DIC (0.37 mL).

The polymer-bound products were precipitated with the addition of *t*-butyl-methyl ether and recrystallized from ethanol. The solid was filtered off to give 2.60 g crude product. Characteristic ^{13}C-NMR ($CDCl_3$) signals: δ 20.51, 20.58, 20.62 ($COCH_3$), 36.72, 36.91 ($OCCH_2CH_2NH$), 46.89, 47.18 (*CH*Fmoc), 52.05 (C-2" and C-2 of 2-amino glucuronic acids), 55.95, 58.90 (OCH_3), 88.45 (C-1, β-azido-glucuronic acid), 98.46, 98.96, 99.00 (C-1, methyl a-glucosides), 102.03 (methyl α-glucoside), 156.10 (*CO*Fmoc).

Treatment of the crude product with a catalytic amount of sodium methoxide resulted in simultaneous cleavage of the saccharo-tripeptides from the polymer carrier and removal of the acetyl and benzoyl protecting groups affording the Fmoc-protected methyl ester

derivatives of the saccharo-tripeptides. After neutralization the solution was concentrated and the residue was subjected to column chromatography (toluene-10% water in methanol-acetone, 2:1:1) to separate the following products.

Methyl {methyl 2-deoxy-2-[3-(3-fluorenylmethoxycarbonylamino-benzamido)-benzamido]-α-D-glucopyranoside} uronate (124), ^{13}C-NMR ($CDCl_3$): δ 46.76 (*CH*Fmoc), 52.64, 53.69 (C-2", OCH_3), 55.66 ($COOCH_3$), 67.45 (CH_2Fmoc), 70.52, 70.90, 72.38 (C-3",4",5"), 98.92 (C-1"), 119.71, 125.15, 127.03, 127.66, 128.41, 129.87 (*Ph*), 133.33, 133.47, 137.28, 141.04 (q, *Ph*), 156.30 (*CO*Fmoc), 165.30, 168.11, 170.40 (2x*CO*NH, *CO*Fmoc, $COOCH_3$).

Methyl {methyl 2-deoxy-2-[3-(N-fluorenylmethoxycarbonyl-β-alanyl)-3-amino-benzamido)-α-D-glucopyranoside} uronate (125), ^{13}C-NMR (CD_3OD): δ 37.92, 38.14 ($OCCH_2CH_2NH$), 48.29 (*CH*Fmoc), 52.81, 55.62, 56.07 (OCH_3, $COOCH_3$, C-2"), 67.72 (CH_2Fmoc), 71.92, 72.84, 73.61 (C-3",4",5"), 100.24 (C-1"), 120.28, 120.82, 124.06, 124.27, 126.06, 128.04, 128.66, 129.84, 129.92 (*Ph*), 136.20, 139.81, 142.46, 145.14 (q, *Ph*), 158.69 (*CO*Fmoc), 170.33, 171.64, 172.23 (2x*CO*NH, *CO*Fmoc, $COOCH_3$).

Methyl {methyl 2-deoxy-2-[3-(methyl 2-deoxy-2-(9-fluorenylmethoxycarbonyl-amino)-α-D-glucopyran uronamido)-3-amino-benzamido]-α-D-glucopyranoside] uronate (126), ^{13}C-NMR (CD_3OD): δ 48.07 (*CH*Fmoc), 52.75, 55.09, 56.03, 59.85 (C-2,2", OCH_3, $COOCH_3$), 62.64 (CH_2Fmoc), 71.67, 72.38, 73.17 (C-3,3",4,4",5,5"), 99.85 (C-1,1"), 119.83, 120.56, 123.77, 124.02, 125.85, 127.76, 128.40, 129.68 (*Ph*), 135.71, 139.45, 142.12 (q, *Ph*), 171.40 (2x*CO*NH, *CO*Fmoc, $COOCH_3$).

Methyl {methyl 2-deoxy-2-[3-(methyl 4-deoxy-4-(9-fluorenylmethoxycarbonyl-amino)-β-D-glucopyran uronamido)-3-amino-benzamido]-α-D-glucopyranoside] uronate (127), ^{13}C-NMR (CD_3OD): δ 48.18 (*CH*Fmoc), 52.65, 55.44, 56.05 (OCH_3, $COOCH_3$, C-2"), 99.99 (C-1"), 105.21 (C-1), 168.66, 169.96, 171.54 (2x*CO*NH, *CO*Fmoc, $COOCH_3$).

Methyl {methyl 2-deoxy-2-[3-(1-azido-1-deoxy-β-D-glucopyran uronamido)-3-amino-benzamido]-α-D-glucopyranoside] uronate (128), ^{1}H-NMR (CD_3OD): δ 3.28 (t, 1H, H-2), 3.42, 3.81 (2s, 2x3H, OCH_3, $COOCH_3$), 3.51 (t, H-3), 3.67 (t, 2H, H-4,4"), 3.88 (dd, 1H, H-3"), 4.05, 4.13 (2d, 2H, H-5,5"), 4.19 (dd, 1H, H-2"), 4.70 (d, 1H, H-1 $J_{1,2}$=8.7 Hz), 4.87 (d, 1H, H-1" partially covered by HOD, $J_{1",2"}$≈ 4 Hz), ^{13}C-NMR (CD_3OD) δ 52.88, 55.56, 56.14 (C-2", OCH_3, $COOCH_3$), 71.93, 72.60, 72.80, 73.61, 74.21, 77.49, 78.96 (C-2,3,4,5,3",4",5"), 92.24 (C-1), 100.25 (C-1"), 120.80, 124.70, 124.83, 130.01 (*Ph*), 136.12, 139.12 (q, *Ph*), 169.09, 170.28, 171.80 (2x*CO*NH, $COOCH_3$).

Synthesis of a 15 component saccharo-tripeptide library

MeOPEGyl {methyl 3,4-di-O-acetyl-2-deoxy-2-[2-(9-fluorenylmethoxycarbonyl)-amino-benzamido]-α-D-glucopyranoside} uronate (129), MeOPEGyl {methyl 3,4-di-O-acetyl-2-deoxy-2-[3-(9-fluorenylmethoxycarbonyl)amino-benzamido]-α-D-glucopyranoside} uronate (122), and MeOPEGyl {methyl 3,4-di-O-acetyl-2-deoxy-2-[4-(9-fluorenylmethoxy-carbonyl)amino-benzamido]-α-D-glucopyranoside} uronate (130).

Composition 119 (13.03 g) was deprotected as described earlier to give 120 (11.62 g, 93 %), which was coupled in a mixture of THF (75 mL) and dichloromethane

(55 mL) with a mixture of 2-(9-fluorenyl-methoxycarbonyl)amino-benzoic acid (0.27 g), 3-(9-fluorenylmethoxycarbonyl)amino-benzoic acid (0.27 g),), 4-(9-fluorenyl-methoxycarbonyl)amino-benzoic acid (0.27 g) in the presence of HOAT (0.36 g) and DIC (0.68 mL). After working up the reaction as previously described, 12.87 g (95%) of the title product was isolated.

The above mixture of **122, 129,** and **130** (12.87 g) was deprotected as described earlier to afford the free aminobenzamido derivatives, which were coupled in a mixture of THF (50 mL) and dichloromethane (25 mL) with the following carboxylic acids:
(0.223 g), **19** (0.268 g), **40** (0.268 g), 3-(9-fluorenylmethoxycarbonyl)amino-benzoic acid (0.151 g), and N-9-fluorenylmethoxycarbonyl-β-alanine (0.130 g) in the presence of HOAT (0.34 g) and DIC (0.81 mL). The polymer was precipitated with the addition of *t*-butyl-methyl ether and recrystallized from ethanol. The solid was filtered off to give the crude product.

The saccharo-tripeptides were removed from the polymer by treating the crude product with a catalytic amount of sodium methoxide, to obtain the N-Fmoc, CO_2Me protected derivatives of the saccharo-tripeptides. The solution was evaporated and the residue was subjected to column chromatography (toluene-10% aq. methanol-acetone, 2:1:1) to obtain a mixture of the following products.

Methyl {methyl 2-deoxy-2-[2- (**131**), 3- (**124**), and 4-(3-(9-flourenylmethoxycarbonyl)amino-benzamido)-amino-benzamido]-α-D -glucopyranoside} uronate (**132**),

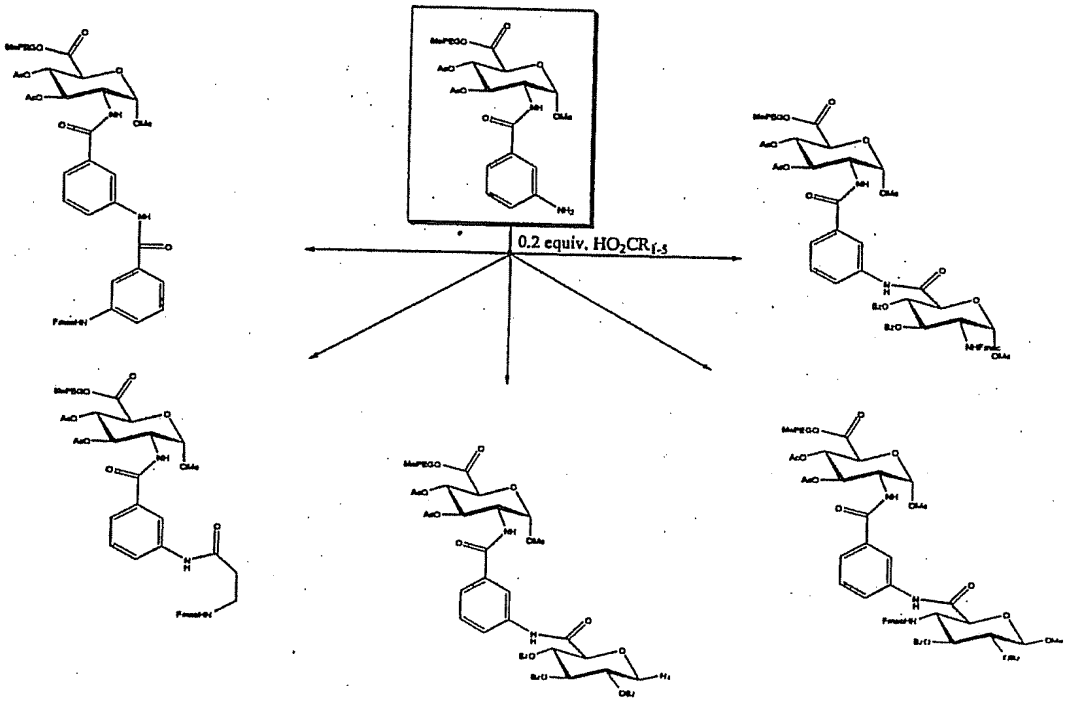
Methyl {methyl 2-deoxy-2-[2- (133), 3- (125), and 4-(N-(9-flourenylmethoxycarbonyl)-β-alanyl)-amino-benzamido]-α-D-glucopyranoside} uronate (134),

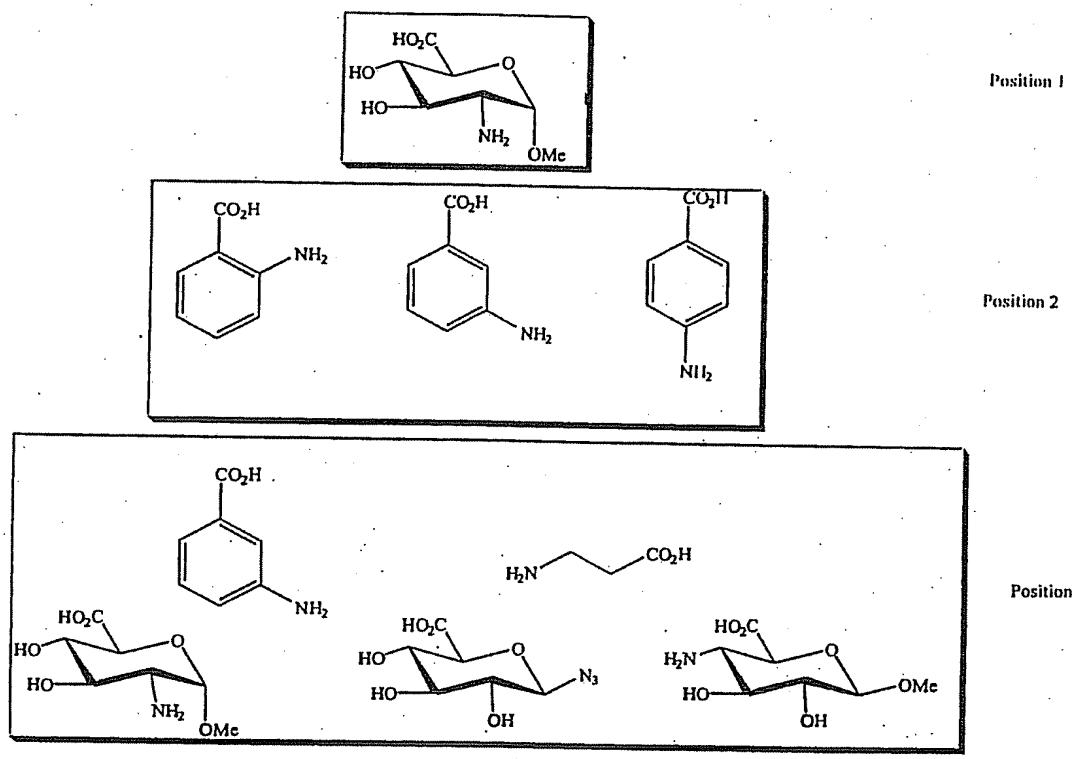
Methyl {methyl 2-deoxy-2-[2- (135), 3- (126), and 4-(methyl 2-deoxy-2-(9-fluorenylmethoxycarbonyl)amino-α-D-glucopyran uronamido)-amino-benzamido]-α-D-glucopyranoside} uronate (136).

Methyl {methyl 2-deoxy-2-[2- (137), 3- (127), and 4-(methyl 4-deoxy-4-(9-fluorenylmethoxycarbonyl)amino-β-D-glucopyran uronamido)-amino-benzamido]-α-D-glucopyranoside} uronate (138).

Methyl {methyl 2-deoxy-2-[2- (139), 3- (128), and 4-(1-azido-1-deoxy-β-D-glucopyran uronamido)amino-benzamido]-α-D-glucopyranoside} uronate (140).

Combinatorial synthesis of saccharopeptides





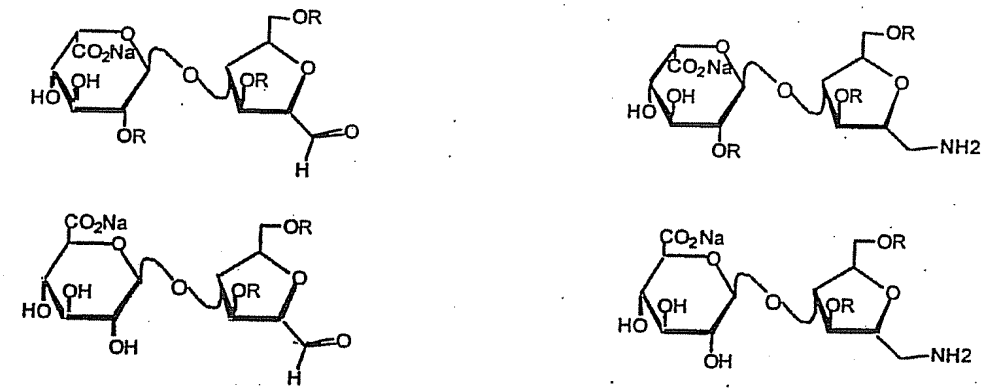
Example 21

Preparation of heparin-derived sacchero amino acids Disaccharides from HONO depolymerization of beef lung heparin

Beef lung Heparin (3 g) was dissolved in 100 ml of water and cooled to 0°C. A solution of N HONO was prepared by treating a N solution of $NaNO_2$ (3.5 g/100mL) at 0°C , with 20 g of Dowex IR 120 (H^+), and then filtering. The heparin solution and HONO solutions at 0°C were combined and reacted for 5h at 0°C . The reaction was then neutralized by the addition of 1N NH_4HCO_3, frozen and lyophilized. Excess ammonium bicarbonate was removed by twice dissolving in 100 ml water and lyophilizing.

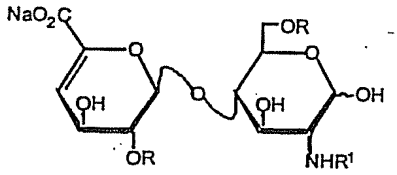
Saccharo amino acid preparation

The disaccharides isolated from glycosaminoglycans can then be converted into saccharo amino acids by reductive amination of the anhydroaldose group with ammonium salts and sodium cyanoborohydride to yield disaccharide glycoaminoacids with a uronic acid residue linked to a terminal 1-amino 2,5 anhydroalditol residue.

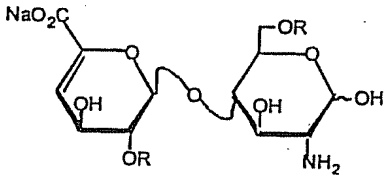


The disaccharide mixture residue was dissolved in 100 mL of 1N ammonium acetate pH8. To the stirring solution, 4g of sodium cyanoborohydride was carefully added. After reacting for ~20h, 1.5 mL of glacial acetic acid was added to destroy excess sodium cyanoborohydride. The reaction was then neutralized with saturated ammonium bicarbonate to PH~7 and lyophilized, redissolved in 1N Na OAc solution (200 mL) and precipitated with ethanol (800 mL). The precipitate was collected and dried to give an oligosaccharide mixture of >95% heparin derived 1-amino-anhydromannitol disaccharide.

Heparin-derived Glycoaminoacids by Enzymatic Depolymerization of heparin



Heparin-derived Disaccharide



Heparin-derived Glycoaminoacid

R = H or SO_3Na
R^1 = SO_3Na or Ac

Disaccharide generation

Methods for the preparation of disaccharides from heparin by enzymatic depolymerization of heparin have been described (A. Horne et al Carbohydr. Res. 225 (1992) 43-57, and M. Ragazi et al J. Carbohydr. Chem. 12 (4&5) 523-535 (1993)). Essentially, Heparin (1g) is dissolved at 1-2% w/v in a suitable buffer such as 0.1M sodium acetate pH 7.0, containing 1 mM calcium chloride. The enzyme preparation (~250 units) is added and allowed to react. The generation of the UV absorbing 4,5-unsaturated uronic acid group was monitored until an absorbance plateau was reached. The solution was then lyophilized to give a mixture of disaccharide and larger fragments of heparin. The disaccharides are isolated from the mixture by size exclusion chromatography (A. Horne et al Carbohydr. Res. 225 (1992) 43-57) or by ion-exchange chromatography (US Patent 5,145,956).

For size exclusion chromatography, the fragments are dissolved in a minimal volume of water, and applied to a BioGel P6 column equilibrated in 0.5 M ammonium bicarbonate. The fraction containing higher molecular

weight components were eluted and then the disaccharides were eluted and isolated by repeated lyophilization.

Glycoaminoacid Preparation

The N-sulfated or N-acetylated disaccharides obtained are then respectively converted to the desired glycoaminoacids by N-desulfation using the method of Y. Inoue and K. Nagasawa, (Carbohydr. Res. (1976) 46, 87-95) or by N-deacetylation using the method of Shaklee and Conrad (Biochem. J., (1984) 217; 187-197).

Example 22

Preparation of (2,3,4-tri-O-acetyl-α-L-fucopyranosyl) acetic acid (141)

To a stirred mixture of 1-C-allyl-1-deoxy-2,3,4-tri-*O*-acetyl-α-L-fucopyranose (10.0 g , 31.9 mmole, in a solvent mixture of acetonitrile - carbon tetrachloride - water (80 ml: 80 ml: 120 ml), 28.0 g (131.2 mmole) of sodium periodate was added, followed by ruthenium trichloride hydrate (145 mg). The reaction became exothermic after 10 minutes and was stirred overnight at room temperature. This mixture was diluted with water (300 ml) and extracted with dichloromethane (2 x 300 ml). The combined organic layer was washed with water (100 ml) and concentrated. The residual oil was dissolved in ethyl acetate (200 ml) and extracted with saturated sodium bicarbonate (30 ml). The organic layer was washed again with water (20 ml) which was combined with the sodium bicarbonate solution extracts. This combined aqueous extract was acidified with 6N

hydrochloric acid solution to pH 1 and extracted with dichloromethane (2 x 200 ml). The combined organic extracts were washed with water (100 ml), followed by saturated sodium chloride solution, dried over sodium sulfate, filtered and concentrated to give 6.9 g (20.8 mmole, 65%) of (2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)acetic acid (141) which was used without further purification.

(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)acetic acid N-hydroxysuccinimide ester (142)

A sample of 141 (1.97 g, 6.16 mmol) was dissolved in dichloromethane (25 ml), *N*-hydroxysuccimide (NHS, 1.0 g, 8.69 mmol) was added to the solution, and the solution was warmed to dissolve the NHS. Dicyclohexylcarbodimide (DCC, 1.41 g, 6.83 mmol) was dissolved in dichloromethane (5 ml) and added to the reaction mixture with stirring. After 5 hours, the reaction mixture was cooled to 4° C, filtered and evaporated. The syrupy residue was taken up in ethyl acetate (50 ml), filtered and washed with water (2 x 25 ml). The ethyl acetate layer was dried over anhydrous sodium sulfate, filtered and evaporated. After drying under high vacuum 2.5 g (94%) amorphous white 142 was obtained.

Coupling of (2,3,4-tri-O-acetyl-α-L-fucopyranosyl)acetic acid N-hydroxy-succinimide ester (142) to heparin-derived glycoamino acid disaccharides

Heparin-derived glycoaminoacid disaccharide (from example 21), (250 mg) was dissolved in 2 mL of 5% sodium bicarbonate solution, and diluted with 2mL of DMF. A

solution of the NHS-C-Fucoside in DMF (0.75g in 1.5 mL) was added in 3 portions of 500 ml at reaction time 0, 6, and 18h. After 24h, the reaction was extracted with methylene chloride to remove excess C-Fucoside. The aqueous layer was the lyophilized to yield the N-(C-fucosyl)acetyl disaccharide derivatives.

N-Deacetylated colominic acid (143)

Colominic acid (250mg) was N-deacetylated by refluxing overnight in 2.0 N sodium hydroxide containing 10 mg $NaBH_4$. After neutralization to pH ~ 8 with 20% acetic acid the solution was filtered and dialysed. Yield: 225 mg of product

N-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)acetylated, N-deacetylated Colominic acid (144)

N-Deacetylated colominic acid (143) (50mg) was dissolved in 2 mL of water, and diluted with 1.0 mL DMF. A solution of 142 was prepared in DMF (250mg in 1 mL DMF). Aliquotes of C-fucoside reagent were added to the N-deacetylated colominic acid solution at reaction times of 0, 4h, and 20h. After a total of 24h reaction time, the reaction was twice extracted with CH_2Cl_2 and the aqueous layer was dialyzed and freeze dried to give 52 mg of product.

N-(α-L-fucopyranosyl)acetylated, N-deacetylated Colominic acid (145)

Composition 144 was disolved in 01.M K_2CO_3 (10 mL) and stirred for 2h at 40C. The solution was dialyzed and lyophilized to yield 40 mg of white solid product. 1H-NMR shows colominic acid peaks, the CH_2 resonances of the CH_2CO-linker and loss of acetate peaks.

4-N-(α-L-fucopyranosyl)acetyl Neuraminic Acid (146)

Composition 145 is dissolved in 9 mL of H_20, diluted by adding 1N HCl (10 mL) and allowed to stir for 4h at 40 C. The reaction solution was then frozen and lyophilized to give the product.

Example A

Selectin Binding Assays

The saccharopeptides of structural formula I can be tested for their ability to bind to a selectin receptor and/or block the binding site of the receptor and thereby prevent a natural ligand from binding to the selectin receptor (Foxall et al, The Journal of Cell Biology (1992) 117:895). A generalized procedure for testing the ligands is given below.

An ELISA assay is preferably used which consists of the following three steps:

1. 2,3 sLex glycolipid (25 picomol/well) was transferred into microtitre wells as solutions and evaporated off. Excess, which remained unattached, was washed off with water. The wells were blocked with 5% BSA at room temperature for an hour and washed with PBS containing 1 mM calcium.

2. Preparation of "multivalent" receptor of the Selectin-IgG chimera was carried out by combining the respective chimera (1 g/mL) with biotin labelled goat F(ab')2 anti-human IgG (Fc specific) and streptavidin-alkaline phosphatase diluted 1:1000 in 1% BSA-PBS (1 mM calcium) and incubating at 37°C for 15 min. This allowed the soluble multivalent receptor complex to form.

3. Potential inhibitors such as saccharopeptides of structural formula I were allowed to react with the soluble receptor at 37°C for 45 min.
This test assumes that optimal binding, between the soluble phase receptor complex and the inhibitor (non-natural ligand), would occur within this time frame. This solution was placed in the microtitre wells that were prepared in step 1. The plate was incubated at 37°C for 45 minutes to allow the soluble receptor to bind to its natural ligand. In the presence of a strong inhibitor only a few receptors would be free to bind to the microtitre plate coated with the natural ligand. The positive control was the signal produced by the soluble receptor when it was allowed to react with the natural ligand in the microtitre wells in the absence of any inhibitor. This was considered 100% binding. The signal produced by the receptor that was previously treated with an inhibitor (recorded as O.D.), was divided by the signal produced by the positive control and multiplied by 100 to calculate the % receptor bound to the well in the presence of the inhibitor. The reciprocal of this is the % inhibition. Shown below in

Table 1 are the results of representative compounds of the present invention tested in this selectin ELISA assay. The numbers listed are IC_{50} measured in mM. Some assays were run with a sensitivity cutoff of 74 mM and some with a cutoff of >1 mM.

Table 1

Selectin binding inhibiton data of saccharopeptides

	Selectin E	ELISA L	IC_{50} (mM) P
53β	>1.0	2.6	0.3
57	>1.0	2.4	0.075
60	>1.0	>1.0	0.206
72	>1.0	0.245	0.159
74	>1.0	0.2	0.213
93	>1.0	0.137	0.237
73	>1.0	0.146	0.442
86	>1.0	0.153	0.029
90	>1.0	0.503	0.855
56	>1.0	1.0	0.609
52α	>1.0	0.963	>1.0

Example B

Selectin Cell-Based Assay

Chinese Hamster Ovary (CHO) cells were transfected by electroporation with plasmids CDM8-E-selectin or CDM8-P-selectin (containing the cDNA for the full-length E- or P-selectin, respectively) and pSVneo, and selected by resistance to neomycin. Individual cells were cloned and/or selected by flow cytometry for selectin expression using monoclonal antibodies to E- or P-selectin.

Plates were prepared as follows:
Ninety-six well Corning plates were coated with 0.2% gelatin. Plates were seeded with either $5x10^4$ cells/well or $3x10^4$ cells/well and grown for either 2 or 3 days. Cells seeded at lower density on Friday will be ready for assay on Monday. The monolayer was rinsed with PBS. Then the cells were fixed with 50µl of 0.5% Paraformaldehyde for 20 minutes. The plates were then rinsed with PBS and blocked with 1% BSA/PBS, 100 µl/well, 20-30 minutes at room temperature. The plates are washed with PBS just before adding compounds.

HL-60 Cell Preparation Was Made As Follows:
HL-60 cells were counted and $7.5x10^6$ cells/plate were removed. The cells were washed by filling a 50 ml centrifuge tube with PBS (no more than 20 ml of cells/50 ml tube). The cells were resuspended at $2x10^6$/ml (7.5 ml for 2 plates). Then BCECF-AM [10 mM stock] at 5µM, 1/2000 dilution was added. The cell preparation was incubated for 30 minutes at 37°C. The tube was filled with PBS to wash, then it was centrifuged as before, and decanted. The cells were pelleted at 1000 rpm for 10

min. The cells were resuspended at $1.5x10^6$cells/ml (10 ml).

Compounds were tested at various concentrations, beginning with a 1:5 dilution. 40 µl of compound is added to quadruplicate wells, followed by 40µl of cells. The suspension is rotated at 50 rpm for 20 minutes at room temperature. Unbound cells are removed or flicked. The mixture is washed 2X with PBS. Then 75 µl of lysis buffer (100 ml TRIS, pH 9.5, 2% Triton S100) is added. The control is 10 µl of labelled cells mixed with 65 µl of lysis buffer. The excitation fluorescence is read at 485 nm, the emission fluorescence is read at 530 nm with a gain of 60 on the cytofluor. A decrease in fluorescence indicates inhibition of adhesion of the cells to the monolayer.

Example C

Selectin Rolling Assay: Effect of Saccharopeptides on Neutrophil Attachment to Selectins

Neutrophils roll along vessel walls, attach to the vessel, and then migrate into tissues at sites of acute inflammation. Selectins mediate the rolling and attachment of neutrophils. Thus, inhibition of neutrophil attachment to selectins, indicates activity as a cell adhesion inhibitor and as an anti-inflammatory.

Adhesion of leukocytes or HL-60 cells to P- and E-selectin under flow conditions in the presence of saccharopeptide is measured according to the methods described by Patel, *et al.* J. Clin. Invest. (1995) 96:1887-1896.

Adhesion of leukocytes or HL-60 cells to P- and E-selectin under flow conditions is assayed as follows. Fluid shear stresses present in the microvasculature are simulated in a parallel-plate flow chamber. Jones, *et al.*, Biophys. J. (1994) 65:1560-1569; Moor, *et al.*, J. Cell. Biol. (1995) 128:661-671. Leukocytes (10^6/ml) in HBSS/0.5% HSA are perfused through the chamber at the desired wall shear stress. Leukocytes rolling is allowed to equilibrate for 4 min. on E- or P-selectin expressing CHO cells or IL-1β, TNFα or IL-4 stimulated human endothelial cells and for 8 min. on selectin-coated plastic before data acquisition. Experiments comparing control and test leukocytes are performed in parallel chambers on the same culture dish. Leukocyte interactions are visualized with a x40 objective (field of view of 0.032 mm^2) using phase- contrast video microscopy. Interactions are quantified using a computer imaging system (Sun Microsystem, Mountain View, CA; Inovision, Durham, NC). The number of adherent or rolling leukocytes is measured by digitizing image frames and determining the number of cells that are firmly adherent or rolling as described by Jones, *et al.* supra. Detachment of leukocytes is determined by allowing leukocytes to adhere to the surface under static conditions then initiating flow at a wall shear stress of 1 dyn/cm^2. The wall shear stress is increased incrementally every 30s and the number of leukocytes remaining adherent is determined. All experiments are performed at 22°C unless indicated otherwise. In certain experiments, cells are preincubated for 10 min

with inhibitor rolling is assayed in the continuous presence of the inhibitor.

Example D

bFGF ELISA

The saccharopeptides of the present invention may be assigned for inhibition of binding to bFGF according to the method of Foxall et al. Anal. Biochem (1995) 231:366-373.

In brief, basic FGF is diluted to 2 µg/ml in 0.1 M carbonate buffer, pH 9.6. Fifty microliters is placed in wells of a microtiter plate (Probind, Falcon, Lincoln Park, NJ or Immulon 4, Dynatech Laboratories, Chantilly, VA) and allowed to absorb at 4 C overnight. The plate is washed three times by dipping and flicking in PBS containing 0.02% Tween 20 (PBST). The wells are blocked with 1% bovine serum albumin (BSA) in PBS at room temperature for 1 h and the plate is washed as above.

A titration of bGFG adsorbed onto the microtiter wells is run to ascertain that the wells are maximally coated. Triplicate wells are coated with 50 µl of bFGF at 0.5, 1, 1.25, 1.5, 1.75, and 2 µg/ml and blocked as described above. Biotinylated HS, 50 µl at 1 µg/ml, is added to each well and assayed as described below.

For the assay to measure direct binding at various concentrations, biotinylated HS or heparin is diluted to 1 µg/ml in 1% BSA-PBST and then serially diluted 10-fold. For the time-course assay, the biotinylated compounds are diluted to 100 ng/ml in BSA-PBST. For competition assays, biotinylated HS is diluted to 2 µg/ml in BSA-PBST. Compounds tested as potential inhibitors of binding are

diluted at 2 x their final concentrations in the above diluent and equal volumes of dilutions and labeled HS are combined. Fifty microliters is added to triplicate wells and binding is allowed to proceed for 45 min at 37 C. The plate is washed as above and 50 µl of streptavidin-alkaline phosphatase diluted 1:5000 in BSA-PBST is added. The plate is incubated for 45 min at 37 C. The plate is washed as above and an additional 3X with distilled water. Substrate for alkaline phosphatase, pNPP, is prepared in 1 M diethanolamine buffer as follows. To 35 ml distilled water, 5 mg of $MgCl_2$ and 4.85 ml of diethanolamine are added. The pH is adjusted to 9.8 with 6.5 ml of 1 N HCl and the volume adjusted to 50 ml with water. The buffer is stored in the dark for not more than a week. Just prior to use, pNPP, 5-mg tablets, is dissolved to provide a 1 mg/ml solution and added at 50 µl/well. Color is allowed to develop for 1 h at room temperature in the dark. Optical density is read at 405 nm in a Molecular Devices plate reader (Menlo Park, CA).

Color development as a result of alkaline phosphatase action on pNPP is allowed in two experiments to determine that OD development is linear. In one experiment, an entire binding curve is read at 1 and 2 h. In the second experiment, ODs of a plate with several inhibition curves are read at 20, 30, 40, 50, and 60 min.

Example E

Effect of Saccharopeptides on Cell Binding and Proliferation

The effect of compositions 98 on the binding of RO-12 UC cells to bFGF coated micro-titer wells was

determined as described by Ishihara, M., et al., Anal Biochem (1992) 202:310-315. (Also see U. S. Patent No. 5,296,471, issued Mar 22, 1994). Bound cells are readily quantitated as total protein. Heparin which inhibits RO-12 UC cell binding was run as a positive control.

The assay was run as follows: Fifty microliters of 10 µg/ml human recombinant bFGF was added to wells of a 96-well tissue culture plate and incubated overnight at 4°C. The wells were aspirated with PBS to remove any unbound bFGF, rinsed twice with PBS, and subsequently incubated with PBS containing 5% (v/v) fetal bovine serum for 1 hour at room temperature. RO-12 UC cells were suspended at a density of 3 x 10^6 cells/ml in PBS containing 5% fetal bovine serum. To this mixture was added the desired amount of sulfated composition, or heparin. They were made up in PBS plus 2.5% fetal bovine serum. A control was also run, containing only PBS plus 2.5% fetal bovine serum. Next, 100 µl of the cell suspension was immediately added to the microtiter wells, and incubated for 5 minutes, after which the wells were washed 3 times with PBS. Finally, the amount of cell protein bound to the wells was determined by dissolving the bound cells in 20 µl of 5% SDS and measuring the protein concentration of the cell lysates. BSA was used as the standard.

To extend the effects seen with RO-12 UC cells, a second experiment was conducted. The capacity of the sulfated maltohexaoses to inhibit the proliferation of a bFGF-dependent adrenocortical endothelial (ACE) cell line was determined. This cell line (provided by D.

Gospodarowicz, UCSF) requires either aFGF or bFGF for a proliferative response. Cells were seeded at low density in microtiter wells in the presence of 2 ng/ml bFGF, and growth was determined as total protein after four days in the presence of the sulfated maltohexaoses.

The APTT values of composition 98 were determined as described in U. S. Patent No. 5,296,471. Table 2 lists the results for the UC-PBA assay, the ACE cell growth inhibition assay, and the APTT values respectively.

Composition 98 is comparable to heparin for bFGF binding activity in the UC-PBA assay (IC_{50} 1 µg/mL. Composition 98 is active in the ACE cell growth inhibition assay. The APTT value of composition 98 was significantly lower than that of other compounds of similar size. Therefore, compound 98 should have less tendancy to cuase bleeding and will work as well as heparin in inhibiting binding to bFGF.

Table 2 lists the results of the assay in terms of the A_{550} values

Table 2

Time (h)	53ß	Control
0.5	0.014	0.104
1.0	0.030	0.199
2.0	0.059	0.396

Composition 53ß reduced ß-glucuronidase activity by about 85%.

As shown in Table 2, the saccharopeptides of the instant invention are inhibitors of the ß-glucuronidase, and would be useful in the purification of the enzyme by affinity chromatography using standard methods.

Example F

Chick Chorioallantoic Membrane (CAM) Angiogenesis Assay

Angiogenic activity of saccharopeptides is assayed on chick chorioallantoic membrane according to the method of Cassellot, J. *et al.*, J. Cell. Phys. (1986) 127:323-329. In brief, the shell-less embryo is suspended in a "sling" made of plastic wrap (Handi-Wrap, Dow Chemical Co.) held in a styrofoam drinking cup with a rubber band Chicken eggs 60-72 hours after fertilization are cracked into the plastic wrap sling, covered with a sterile plastic top from a 100-mm culture dish, and placed in a humidified 38°C incubator. Nine days after fertilization, 40µl agarose pellets containing test substances are placed on the CAM which occupies an area of 30-40 cm^2 at this stage. Pellets are prepared by rapidly combining 20µl of 6% aqueous low melting point agarose (Sigma type VII) with an equal volume of test substance at 37°C, thereby avoiding heating of the test material. The pellets are placed at 4°C for several minutes to solidify. This technique results in >50% survival of the embryos, allows direct visual and photographic monitoring of the experiment, and permits 5-8 samples to be tested on a single egg. Scoring is done in single blind fashion on day 3 or 4 after adding test substance. Histological sections of positive responses are routinely prepared and examined

for the presence of inflammatory cells, since inflammation of the CAM could also result in a positive response. Pellets that had elicited an obvious inflammatory response, as evidenced by the clear translucent pellet becoming white and opaque are not scored. Inhibition of angiogenesis can be an important aspect of wound healing, treating inflammation, and inhibiting tumor growth.

Example G

Chronic Inflammation: Guinea Pig Asthma Model

The procedure for assessing saccharopeptides in the guinea pig asthma model is described by Ito, *et al.* Int. Arch. Allergy Immunology (1996) 109:86-94. In brief, the animals are sensitized with 0.5 ml of 5% ovalbumin subcutaneously and .5 ml intraperitoneally. A booster injection is given 7 days later. Seven or eight days after the booster, oralbumin (10 mg/ml) is inhaled using a nebulizer undercover of an H_1 antagonist, mepyramine (10 mg/kg) i.p.). Thirty minutes later the animals are challenged with 1% ovalbumin for 3 minutes. A second challenge is administered 7 days later, and the experiments are performed 7 or 8 days after this. The mepyramine is used before each antigen challenge.

As an index of bronchoconstriction, specific airway resistance is determined on a breath-by-breath basis in a double- chamber plethysmograph with a respiratory analyzer (Non-Invasive Model, Buxco, Inc., Sharon, Conn., USA) and data logger (Model OA-16, Buxco) Specific Airway resistance is measured before and 0-6h after challenge.

Airway responsiveness is determined by measuring airway resistance to doubling the concentration of methacholine. As an index of bronchoconstriction to methacholine, respiratory resistance is automatically measured by a forced oscillation technique using Animal-asto (TMC-2100. Chest-M1, Japan) with a multinebulizer. In brief, guinea pigs are placed inside a body plethysmograph, and a 30-Hz sine wave oscillation is applied to the animal body surface. The flow rate through the mask and box pressure is measured by a differential pressure transducer. The 3-Hz components of mask flow and box pressure are extracted by a lock-in-amplifier. The resistance is calculated. A reduction in airway resistance is indicative of anti-inflammatory activity.

Methacholine (32-4,096 μg/ml) or saline aerosol is generated using an ultrasonic nebulizer driven by compressed air. Saline is inhaled for 1 min, and increasing concentrations of methacholine are inhaled for 1 min each at intervals of 1 min. The minimum provocative concentration of methacholine at which resistance exceeded 200% of the baseline value of individual animals is calculated and expressed as PC_{200} (μg/ml). PC_{200} values are determined 1h before (pre), and 4 and 24h after antigen inhalation.

Guinea pigs are anesthetized with pentobarbital (30 mg/kg i.p.) before each bronchoalveolar lavage. Tracheas are cannulated by a disposable intravenous catheter, 3-Fr-size (ATOM Co, Tokyo, Japan), and the airway lumens are washed three times with equal portions of 0.9% saline at 37°C (10 ml/kg); typically, more than

75% of the fluid is recovered. The BALF collected from each animal is immediately placed in an ice bath and centrifuged (150 g for 10 min at 4°C). The precipitants are used to evaluate cell component and number.

Cell pellets obtained after centrifugation of BALF are resuspended in 4 ml HBSS (Hank's balanced solution) and total cell counts are performed using a standard hemocytometer. Differential cell counts are performed on smears fixed in methanol and stained with Wright solution. A minimum of 500 cells per smear are counted by light microscopy under oil immersion (x1,000). The proportion of each cell population is expressed as a percentage of total cells, and this ratio, together with the total cell count is used to calculate the total number of each cell type. A reduction in eosinophils and/or neutrophils is indicative of anti-inflammatory activity.

Example H

Guinea Pig Eosinophilia Model

Using a similar protocol to that used in the asthma model described above, the effect on antigen-induced granulocyte acculation in bronchoalveolar lavage fluid (BALF) of actively sensitized guinea pigs is assayed.

In brief, Hartley guinea pigs are sensitized with 50 mg/ml ovalbumin twice at day -14 and -7. Saccharopeptides are intravenously injected 1 hour before and 1 hour after inhalation of the aerosol ovalbumin (10 mg/ml) for 6 min at day 0. Five minutes before the ovalbumin challenge, .001% salbutamol is inhaled for 5 minutes. Lavage fluid is collected 4 hr. after the inhalation of antigen. The number of eosinophils and neutrophils is counted. The density distribution of the eosinophils is measured by Mycodenz discontinuous density-gradient. Reduced numbers of

eosinophils and neutrophils indicates anti-inflammatory activity.

Example I

Adjuvant-induced Arthritis in Rats

Female Lewis -LEW/CrlBR (Charles River Laboratories) rats weighing 160 - 190 grams are injected in the tail with 0.75 mg *Mycobacterium butyricum* (0.75 mg in 0.1 mL paraffin or light mineral oil) subcutaneously on Day 1. Just prior to tail injection, the hind paw volumes for all rats are determined and the rats distributed as evenly as possible into groups of ten rats each according to total hind paw volume using the LabCat Randomization Program. The assay is performed using various dose levels of the saccharopeptide and one vehicle control group. The vehicle can be methylcellulose at 0.25% concentration. An additional group of ten rats receive indomethacin at 2.5 mg/kg, orally. All animals are dosed orally by gavage subcutaneously, or intraperitoneally, once daily from Day 1 through Day 19. The animals can also be variably dosed, or dosed via intravenous infusion. The hind paw volumes are monitored by water displacement each day post-adjuvant administration.

The volume of the hind paws of the rats are recorded plethysmographically using water displacement via a transducer coupled to the inner well of the edema table. Data are collected via the BUXCO Biosystem for edema software and exported for further statistical analysis. Preliminary calibration of the hardware and signals is performed. The volume reading is reset to

zero and the hind paws are extended and immersed up to the natural hairline. The system is activated to automatically take the readings and advance to the next subject. All data generated is equal to volume (mL) of water displaced. A reduction in hind paw volume is indicative of anti-inflammatory activity.

Example J

Rat Granulomatous Vasculitis Model

Determination of the ability of saccharopeptide glycomimetics to reduce glucan-induced pulmonary granuloma formation

Saccharopeptide compounds are tested in the *in vitro* assay and in an *in vivo* model of pulmonary granuloma formation. These procedures are described more fully in Flory, *et al*. Lab. Invest. (1993) 69:396-404; and Flory, *et al*. Am. J. Path (2995) 146:450-462. Protocols I-III are designed to permit an evaluation of saccharopeptide glycomimetics as protective agents against pulmonary granuloma formation as compared to control animals administered vehicle (saline). The infusion of yeast cell wall glucan (5 mg/kg) followed by morphometric analysis 48 hours later is used in order to assess the protective effect of the glycomimetic. Protocol I permits the assessment of the protective actions of the glycomimetics in that the compounds of interest are administered prior to glucan infusion. In contrast, test groups in Protocols II and III will more closely mimic the clinical setting in that the compound is administered 6 and 12 hours after glucan infusion respectively. Early granuloma development (1-6 hours)

is characterized by the transient influx of neutrophils into the area of glucan deposition. After this time, the monocytes and macrophages that contstitute the final granuloma are recruited. Protocol I will be utilized to determine the ability of the glycomimetics to inhibit the early, neutrophil-mediated phase of granuloma development. Protocols II and III are directed against the monocyte/macrophage phase in an effort to determine the ability of the glycomimetics to influence these cell types in an effort to decrease granuloma formation. Each group (vehicle control and glycomimetic-treated) consists of a minimum of 6 animals found suitable for inclusion in the final data analysis.

Protocol I:

Glucan-induced Pulmonary Granulomatosis:

Male, specific pathogen-free Long-Evans rats weighing 250-300 gm are housed in specific pathogen free rooms with laminar air flow. Pulmonary granulomas are induced by infusion of particulate glucan (5 mg/rat) into the dorsal penile vein of anesthetized animals. At the time of sacrifice, the lungs are slowly instilled with 4% paraformaldehyde (4 ml), followed by routine processing for both light and electron microscopy.

Protocol II:

In Vivo Studies: Interventions with Saccharopeptides:

At the time of glucan infusion, the saccharopeptide is infused *via* the dorsal penile vein. Control rats are treated identically except that vehicle (saline) is substituted for the compound under investigation. The

effect of the saccharopeptide on circulating white blood cell number is determined by staining peripheral blood smears with Wrights-Giemsa type stain followed by a differential cell count.

Protocol III:

Morphometric Analysis of Lung Granuloma Size and Number:

Morphometric analysis of granuloma formation is conducted on lung sections derived from animals sacrificed 48 hours following glucan infusion. Twenty 10x fields are chosen at random from blinded sections and the number and area of granulomas per field recorded. The area of each granuloma is measured using a Sony video image camera coupled to a Macintosh II FX computer with NIH Image 1000 software. The area is converted from pixels^2 to μm^2 by measuring a known area on a hemocytometer to obtain a conversion factor.

Lung MPO (Myelo peroxidase) assays are utilized to determine if the presence of the glycomimetic influences the accumulation of neutrophils within the lungs of animals that have received glucan. If MPO levels are not significantly decreased in treated animals as compared to control, the possibility that the compounds are affecting the migration of the neutrophils from the vasculature in the extravascular compartment is investigated by electron microscopy. Use of EM provides information as to the location of neutrophils (vascular space vs extracellular space).

Lung MPO Assay:

Upon removal, lungs for MPO analysis are immediately frozen in liquid N_2. The tissue is placed in two volumes of homogenization buffer (50 mM sodium phosphate, pH 6.0) and homogenized. The homogenates are centrifuged for 30 minutes (3000 x g, 4°C) and the supernatants removed. MPO activity is determined by measuring the changes in absorbance at 460 nm resulting from the conversion of H_2O_2 in the presence of *O*-dianisidine. The MPO activity is normalized to the weight of the tissue.

Neutrophils are required for full granuloma development. It appears that neutrophil-derived products (H_2O_2) act to increase the expression of the monocyte chemotactic cytokine MCP-1. Thus, inhibiting neutrophil adhesion/accumulation may provide an indirect mechanism of action for the glycomimetics. To determine the effect of these compounds on chemokine expression, immunohistochemistry of lung sections is conducted using polyclonal antibodies against rat chemotactic cytokines.

Immunohistochemical Analysis of MCP-1 Expression:

Lung sections removed from animals sacrificed 6 hours post-glucan infusion are incubated with a rabbit purified IgG raised against recombinant rat MCP-1 (1:250 dilution). Following repeated washings, cells are incubated with a biotinylated goat anti-rabbit secondary antibody (1:1000 dilution). Detection of the primary antibody is accomplished using a Vectastain ABC kit (Vector Laboratories) with three-amino-9-ethyl-carbazole as the substrate. Controls included sections in which the primary antibody is omitted and sections incubated

with an isotype-matched murine IgG1 antibody in place of the primary antibody.

Example J

Assay For Heparanase Inhibition Activity of Heparin Mimetics Quantitation of Heparanase Activity in Soluble Extracts of Rat Hepatoma Cells Using a CPC Precipitation Assay

Using the method described by Lapierre, *et al.*, Heparanase inhibition activity is measured. Glycobiology (1996) 6(3):355-366. The CPC precipitation assay was developed from the observation that heparanase-cleaved HS chains (derived from [^{3}H]pancreatic HS substrate) can be distinguished from uncleaved chains by selectively precipitating the latter with CPC (Oldberg et al., Biochem. (1980) 19:5755; Bame, J. Biol. Chem. (1993) 268:19956). Briefly, this assay is conducted by combining [^{3}H]acetylated pancreatic HS (10 mL, 250 ng, 80,000 CPM) in 200 mM MES, 0.14 M NaCl pH 5.2, with test compound (10 mL) prepared at concentrations of 0-1000 mg/mL in water. All assays are run in triplicate along with heparin as a positive control. Hepatoma soluble extracts (30 mL) diluted to 333 mg/mL in freshly prepared Buffer 1 are added to each tube (with the exception of those used to measure background CPM, i.e., no test compound) at 0°C. The samples are incubated for 20 min in a 37°C water bath, after which heparin (150 mL, 333 mg/mL) is added to terminate the reaction. The 20 min. incubation time was selected after observing that the rate of HS hydrolysis

in this system had begun to decrease by 30 min. It was determined that substrate depletion rather than enzyme inactivation caused the decrease in rate since no increase in the rate of substrate cleavage was observed after the addition of fresh enzyme. Furthermore, preincubating the enzyme at 37°C did not affect the rate of HS hydrolysis. Soluble hepatoma extracts are added to each of the background tubes at this time, after which heparin is added. A 100 mM solution of sodium acetate, pH 5.5 (200 mL), is added to each tube followed by a solution of 0.6% CPC w/v in water (100 mL). The tubes are vortexed and incubated for 1 h at ambient temperature, and then centrifuged for 10 min at 4,000 x g in an Eppendorf 5415C microcentrifuge. Supernatant (400 mL) is carefully removed and assayed for ^{3}H by liquid scintillation counting. To verify that the CPC reagent is not depleted at the highest concentration of heparin or test compound used in the assay, 200 mg/mL of compound is incubated for 20 min with hepatoma extracts and [^{3}H]pancreatic HS substrate at 0°C. There should be no detectable difference between the soluble CPM from these reaction tubes and background CPM. To examine the effects of other lysosomal exohydrolase inhibitors on heparanase activity in this assay, 10 mL of ISMS (1000 mg/mL) and GlcNAc (3000 mg/mL) in water are added to the assay tubes containing the radiolabeled HS substrate and soluble hepatoma extracts (50 mL total volume). Inhibitor concentrations are chosen to inhibit >99% of iduronate 2-sulphatase and -iduronidase activities based upon K_i values for the inhibitors and K_m values of liver iduronate 2-sulphatase for the substrate ISMS, and

of liver -iduronidase for the substrate IMS (Hopwood, (1989) "Enzymes that Degrade Heparin and Heparin Sulfate" in Heparin (Lane and Lindahl eds.) CRC Press, Inc., Boca Raton, FL, pp. 191-227; Freeman and Hopwood, Adv. Exp. Med. Biol. (1992) 313:121.)

Characterization of Heparanase-cleaved [^{3}H]Acetylated Pancreatic HS by Size-exclusion HPLC

The acetylated pancreatic HS samples are incubated with hepatoma soluble extract for 0, 10, 15 and 20 min, and then the reaction is quenched by adding heparin (150 mL, 333 mg/mL) followed by boiling for 10 min. Samples are filtered through 0.2 mm microcentrifuge filter inserts, spun for 10 min at 16,000 x g, evaporated to dryness under nitrogen, and then redissolved in filtered water (20 mL). Sample aliquots (17 uL) are injected onto the HPLC size-exclusion system described previously. Fractions (375 mL) are collected and assayed for [^{3}H] by liquid scintillation counting. [^{3}H]-labeled heparin hexasaccharide standard is chromatographed to determine the time lag between observing the oligosaccharide using the refractive index detector, and elution of the oligosaccharide as measured by liquid scintillation counting. The CPM for each sample time point is normalized to the sample time point with the highest total CPM in order to compare profiles.

Example K

ß-Glucuronidase Inhibition Assay

Composition 53ß was used to measure the ß-glucuronidase activity, and the measurement was

performed in the same manner as that of the conventional method.

The procedure was carried out according to instructions provided in the Sigma kit 325-A. The reagents used were provided in the kit and the proportions of the reagents used is described in the table below:

Table 3

Assay/Reagent	Blank	Control	9ß
Enzyme (µl)	2	2	2
Acetate buffer* (µl)	3	3	3
PGA# (µl)	-	10	10
53ß (50 mM, µl)	-	-	15
Water (µl)	25	15	-

* 0.2M sodium acetate buffer, pH 4.5 at 25°C; # 30mM phenolphthalein glucuronic acid (PGA), pH 4.5 at 25°C.

All components, except PGA, were pre-incubated at room temperature for 30 min, PGA was then added to the mixture. At various time points (0.5, 1 and 2h), 2µl aliquots were removed and added to 1 ml assay diluent, mixed and absorbance read at 550 nm against the blank (A_{550}). The assay diluent is a mixture of acetate buffer (1.44 ml), water (0.96 ml) and AMP (2-amino-2-methyl-1-propanol) buffer (12 ml, 0.1M AMP, pH 11, containing 0.2% sodium lauryl sulfate).

Table 4 lists the results of the assay in terms of the A_{550} values

Table 4

Time (h)	53ß	Control
0.5	0.014	0.104
1.0	0.030	0.199
2.0	0.059	0.396

Composition 53ß reduced ß-glucuronidase activity by about 85%.

As shown in Table 4, the saccharopeptides of the instant invention are inhibitors of the ß-glucuronidase, and would be useful in the purification of the enzyme by affinity chromatography using standard methods.

Example M

Cancer Models

Various cancer models are well-known in the art. The following are examples of some of the many cancer models that can be used.

Subcutaneous Tumor Growth of a Human Pancreatic (CaPan-2) Adenocarcinoma in Nude Mice:

CaPan-2 tumor cells at 3×10^6 cells/mL are injected in a volume of 0.1 mL s.c. in the anterior dorsal region of male Balb/c athymic nude mice (Simonsen Laboratories, Gilroy, CA). Animals (10 animals/treatment group) received a daily s.c administration of 1 µg-100 mg of saccharopeptide or PBS vehicle on day 1 to day 35.

Tumor measurements are performed three times a week and data are evaluated with Analysis of Variance SAS JMP Version 3.

B16-F10 Melanoma Experimental Lung Metastasis Assay:

Female C57BL/6N mice, 4-6 weeks old (Charles River, Raleigh, NC), are injected with 5 x 10^4 B16-F10 melanoma cells in a volume of 0.1 mL intravenously (i.v.) in the tail. Animals are randomly distributed prior to assignment of saline vehicle control and treatment groups. Animals (10 animals/treatment group) received saccharopeptide at 1-100 mg/kg s.c., i.v., oral, or i.p. at a single dose (0.05 mL) or 0.9% saline vehicle on days 0-4 with the first dose occurring one h prior to tumor challenge. Survival times were recorded for animals in the vehicle control and treatment groups. Results were evaluated with Chi-squared statistical analysis.

Effects of Heparin Mimetics on Proliferation of Human CaPan-2 and B16-F10 Melanoma Cells:

In 48-well plastic tissue culture plates (Corning), CaPan-2 cells are plated at 20,000 cells per well in McCoy's 5A media containing 10% fetal bovine serum and L-glutamine while B16-F10 melanoma cells are plated at 2,000 cells per well in DMEM with 10% fetal bovine serum. The media also contains 100 µg/mL of saccharopeptide dissolved in the appropriate vehicle (0.9% saline or PBS) or vehicle alone. Using a Coulter counter, the total number of cells in each well is

determined after 24, 48 and 96 h incubation at 37°C. Six replicates are done for each condition used and the means of total cell counts are determined.

While the present invention is disclosed by reference to the details of above examples, it is to be understood that this disclosure is intended in an illustrative rather than limiting sense, as it is contemplated that modifications will readily occur to those skilled in the art, with the spirit of the invention and the scope of the appended claims.

What is Claimed is:

1. The glycomimetic saccharopeptide compounds of formula I:

$$W-(X)_n-Y-[(X)_n-W-(X)_n-Y]_m-(X)_n-W \qquad (I)$$

wherein

W is independently selected from the group consisting of

a) saccharides;

b) aryl, aralkyl;

c) alkyl of 1 to 8 carbon atoms, optionally substituted with 1-2 substituents selected from the group consisting of lower aryl, lower alkyl, =O, -OR, $-NR'_2$, -SR, $-SO_4R$, $-SO_3R$, -COOR, -alk-COOR; and

d) cyclic-alkyl or 5-7 carbon atoms, heterocyclic alkyl of 5-7 ring atoms and 1-2 heteroatoms selected from the group consisting of N, O, and S, all optionally substituted with 1-5 substituents selected from the group consisting of =O, -OH, -OR, $-NR'_2$, -SR, $-SO_4R$, $-SO_3R$, -COOR, and -alk-COOR;

Y is independently selected from the group consisting of $-NR^3-C(O)-$ and $-C(O)-NR^3-$;

X is a difunctional or polyfunctional group selected from the group consisting of

a) aryl, aralkyl;

b) alkyl of 1-8 carbon atoms, optionally substituted with 1-3 substituents in the alkyl backbone or exo to the backbone selected from the group consisting of lower aryl, lower alkyl, -O-, -NR'-, -S-,

=O, -OH, -OR, $-NR'_2$, -SH, -SR, $-SO_4R$, $-SO_3R$, -COOR, and -alk-COOR;

each n is independently 0 or 1;

each m is independently 0 or an integer from 1 to 99 with the proviso that the total number of W groups is 2-100;

R is -H, or lower alkyl, lower aryl, and lower aralkyl;

R'is independently selected from the group consisting -H, lower alkyl of 1-4 carbon atoms, aralkyl of 2 to 19 carbon atoms, and -C(O)R";

R" is lower alkyl of 1 to 4 carbon atoms; and

R^3 is selected from the group consisting of -H, alkyl of 1-8 carbon atoms, and aralkyl of 5-8 carbon atoms;

and pharmaceutically acceptable salts thereof; with the following provisos:

a) when the total number of W groups is 2, then both W groups may not be 2-amino hexoses;

b) at least one W group is a saccharide; and

c) if the terminal W is a N-acetylglucosamine, it may not be linked through -NHCO- at the anomeric carbon to a natural amino acid.

2. The compounds of claim 1 wherein

m is an integer of 1-5; and

W is independently selected from the group consisting of fucose, 3-amino-3-deoxy glucose, 4-amino-4-deoxy glucose, glucose, galactose, glucosamine, galactosamine, glucuronic acid, galacturonic acid, glucosamine uronic acid, neuraminic acid, maltose, maltotriose, iduronic acid, 2,5-anhydromannitol, mannose, mannuronic acid, and cellobiose.

3. The compounds of claim 2 wherein

m is an integer of 1-2; and

W is independently selected from the group consisting of glucuronic acid, and glucosamine.

4. The compounds of claim 1 wherein

W is selected from the group consisting of maltose, maltotriose, and cellobiose.

5. The compounds of claim 4 wherein

n and m are 1; and

X is independently selected from the group consisting of ethylene glycol, ethylene glycol oligomers, lower alkyl, optionally substituted alkyl, amino acid, and peptides.

6. The compounds of claim 1 having the formula

W' - Y - W" - Y - W'

wherein

each W' is independently selected from the group consisting of saccharides;

W" is selected from the group consisting of

a) aryl, aralkyl,

b) alkyl of 1 to 8 carbon atoms, optionally substituted with 1 to 2 substituents selected from the group consisting of lower aryl, lower alkyl, =O, -OR, $-NR_2'$, -SR, $-SO_4R$, $-SO_3R$, -COOR, -alk-COOR; and

c) cyclic alkyl of 5-7 carbon atoms, heterocyclic alkyl of 5-7 ring atoms and 1-2 heteroatoms selected from the group consisting of N, O, and S, all optionally substituted with 1-5 substituents selected from the group consisting of =O, -OH, -OR, $-NR_2'$, -SR, $-SO_4R$, $-SO_3R$, -COOR, and -alk-COOR; and

Y is -NH-CO-.

7. The compounds of claim 1 having the formula

W' - Y - W' - Y - W"

wherein

each W' is independently selected from the group consisting of saccharides;

W" is selected from the group consisting of

a) aryl, aralkyl,

b) alkyl of 1 to 8 carbon atoms, optionally substituted with 1 to 2 substituents selected from the group consisting of lower aryl, lower alkyl, =O, -OR, $-NR_2'$, -SR, $-SO_4R$, $-SO_3R$, -COOR, -alk-COOR; and

c) cyclic alkyl of 5-7 carbon atoms, heterocyclic alkyl of 5-7 ring atoms and 1-2 heteroatoms selected from the group consisting of N, O, and S, all optionally substituted with 1-5 substituents selected from the group consisting of =O, -OH, -OR, $-NR_2'$, -SR, $-SO_4R$, $-SO_3R$, -COOR, and -alk-COOR; and

Y is -NH-CO-. --

8. The compounds of claim 1 wherein at least one terminal W group is substituted with $-NR_2'$, SO_3R, or -COOR.

9. The compounds of claim 1 wherein the total number of W groups is 2-8.

10. The compounds of claim 10 wherein the total number of W groups is 3-4.

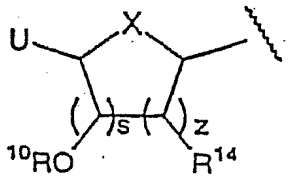
11. The compounds of claim 1 having the formula

$$W^1 - Y - W - Y - W^2$$

wherein

W^1 is selected from the group consisting of $-(C=O)R^{11}$, sialic acid, Kemp's acid, -B, $-SO_3M$, $-OSO_3M$, $-SO_2NH_2$, $-PO_3M'_2$, $-OPO_3M'_2$, $-NO_2$, saturated or unsaturated carboxylic acids of 1 to 4 carbon atoms, optionally substituted with 1 to 2 hydroxyl groups, and esters, and amides thereof;

W^2 is



wherein

U is selected from the group consisting of $-R^9$, $-CH_2OR^{10}$, $-CH_2O$-protecting group, $-COOR^{11}$, $-CON(R^{11})_2$, and -COOM;

R^9 is lower alkyl;

each s is independently selected from the group 1, 2, and 3;

each z is independently selected from the group 1 and 2;

R^{10} is selected from the group consisting of -H, $-R^{11}$, $-SO_3M$, $-(C=O)R^{11}$, $-SO_2NH_2$, $-PO_3M'_2$, $-alk-COOR_{13}$, $-alk-CON(R^{11})_2$ and -O-carbohydrate;

R^{11} is independently selected from the group consisting of -H, lower alkyl, cyclic alkyl of 5 to 6 carbon atoms, heterocyclic alkyl of 4 to 5 carbon atoms and 1 to 2 heteroatoms, lower aryl and lower aralkyl;

R^{13} is selected from the group consiting of R^{11}, and M;

R^{14} is selected from the group consisting of -H, and $-OR^{10}$;

M is selected from the group consisting of Na^+, K^+, Mg^{2+}, and Ca^{2+};

M' is selected from the group consisting of -H, -M, and R^9; and

X is selected from the group consisting of -O-, -S-, $-N(R^{11})-C(R^{11})_2-$, and $-N(R^{11})-$; and

B is a W^2 group containing at least one $-COOR^{11}$, $-CON(R^{11})_2$, -COOM, $-SO_3M$, or $-(C=O)R^{11}_2$ substituent.

12. The compounds of claim 1 wherein there is a total of 4-8 W groups, where 2-4 of said W groups are saccharides, optionally fully or partially sulfated.

13. A method of treating a patient for a condition wherein said condition is selected from the group consisting of Alzheimer's disease, atherosclerosis, inflammation, retinopathy, cancer, infections, and auto-immune diseases comprising the administration of a pharmaceutically effective amount of a compound of formula I:

$$W-(X)_n-Y-[(X)_n-W-(X)_n-Y]_m-(X)_n-W \qquad (I)$$

wherein

W is independently selected from the group consisting of

a) saccharides;

b) aryl, aralkyl;

c) alkyl of 1 to 8 carbon atoms, optionally substituted with 1-2 substituents selected from the group consisting of lower aryl, lower alkyl, =O, -OR, $-NR'_2$, -SR, $-SO_4R$, $-SO_3R$ -COOR, alk-COOR; and

d) cyclic alkyl or 5-7 carbon atoms, heterocyclic alkyl of 5-7 ring atoms and 1-2 heteroatoms selected from the group consisting of N, O, and S, all optionally substituted with 1-5 substituents selected from the group consisting of =O, -OH, -OR, $-NR'_2$, -SR, $-SO_4R$, $-SO_3R$, -COOR, and alk-COOR;

Y is independently selected from the group consisting of $-NR^3-C(O)-$ and $-C(O)-NR^3-$;

X is a difunctional or polyfunctional group selected from the group consisting of

a) aryl, aralkyl;

b) alkyl of 1-8 carbon atoms, optionally substituted with 1-3 substituents in the alkyl backbone or exo to the backbone selected from the group consisting of lower aryl, lower alkyl -O-, $-NR'-$, -S-, =O, -OH, -OR, $-NR'_2$, -SH, -SR, $-SO_4R$, $-SO_3R$, -COOR, and alk-COOR;

each n is independently 0 or 1;

each m is independently 0 or an integer from 1 to 99 with the proviso that the total number of W groups is 2-100;

R is -H, or lower alkyl, lower aryl, and lower aralkyl;

R'is independently selected from the group consisting -H, lower alkyl of 1-4 carbon atoms, aralkyl of 2 to 19 carbon atoms, and $-C(O)R''$;

R" is lower alkyl of 1 to 4 carbon atoms; and

R^3 is selected from the group consisting of -H, alkyl of 1-8 carbon atoms, and aralkyl of 5-8 carbon atoms; and pharmaceutically acceptable salts thereof; with the following provisos:

a) when the total number of W groups is 2, then both W groups may not be 2-amino hexoses;

b) at least one W group is a saccharide; and

c) if the terminal W is a N-acetylglucosamine, it may not be linked through -NHCO- at the anomeric carbon to a natural amino acid.

14. The method of claim 13 wherein said disorder is cancer.

15. The method of claim 14 wherein said cancer is selected from the group consisting of renal carcinoma, colon carcinoma, stomach carcinoma, esophogeal carcinoma, liver carcinoma, breast carcinoma, lung carcinoma, prostate carcinoma, bladder/urinary carcinoma, brain gliomas, lymphomas, ovarian carcinoma, uterin carcinoma, and sarcomas.

16. The method of claim 13 wherein said disorder is inflammation.

17. The method of claim 16 wherein the inflammatory condition is selected from the group consisting of arthritis, inflammatory bowel diseases, psoriasis, reperfusion injury, septic shock, hypovolemic or traumatic shock, acute respiratory distress syndrome, and asthma.

18. The method of claim 13 wherein said disorder is an auto-immune disease.

19. The method of claim 18 wherein said auto-immune disease is selected from the group consisting of systemic lupis erythematosis (SLE), rheumatoid arthritis (RA), scleroderma, and dermatomysitis.

20. A method of inhibiting angiogenesis in a patient in need thereof comprising the administration of a pharmaceutically effective amount of a compound of formula I:

$$W-(X)_n-Y-[(X)_n-W-(X)_n-Y]_m-(X)_n-W \qquad (I)$$

wherein

W is independently selected from the group consisting of

a) saccharides;

b) aryl, aralkyl;

c) alkyl of 1 to 8 carbon atoms, optionally substituted with 1-2 substituents selected from the group consisting of lower aryl, lower alkyl, =O, -OR, $-NR'_2$, -SR, $-SO_4R$, $-SO_3R$, -COOR, -alk-COOR; and

d) cyclic alkyl or 5-7 carbon atoms, heterocyclic alkyl of 5-7 ring atoms and 1-2 heteroatoms selected from the group consisting of N, O, and S, all optionally substituted with 1-5 substituents selected from the group consisting of =O, -OH, -OR, $-NR'_2$, -SR, $-SO_4R$, $-SO_3R$, -COOR, and -alk-COOR;

Y is independently selected from the group consisting of $-NR^3-C(O)-$ and $-C(O)-NR^3-$;

X is a difunctional or polyfunctional group selected from the group consisting of

a) aryl, aralkyl;

b) alkyl of 1-8 carbon atoms, optionally substituted with 1-3 substituents in the alkyl backbone or exo to the backbone selected from the group consisting of lower aryl, lower alkyl -O-, -NR'-, -S-, =O, -OH, -OR, $-NR'_2$, -SH, -SR, $-SO_4R$, $-SO_3R$, -COOR, and alk-COOR;

each n is independently 0 or 1;

each m is independently 0 or an integer from 1 to 99 with the proviso that the total number of W groups is 2-100;

R is -H, or lower alkyl, lower aryl, and lower aralkyl;

R'is independently selected from the group consisting -H, lower alkyl of 1-4 carbon atoms, aralkyl of 2 to 19 carbon atoms, and -C(O)R";

R" is lower alkyl of 1 to 4 carbon atoms; and

R^3 is selected from the group consisting of -H, alkyl of 1-8 carbon atoms, and aralkyl of 5-8 carbon atoms; and pharmaceutically acceptable salts thereof; with the following provisos:

a) when the total number of W groups is 2, then both W groups may not be 2-amino hexoses;

b) at least one W group is a saccharide; and

c) if the terminal W is a N-acetylglucosamine, it may not be linked through -NHCO- at the anomeric carbon to a natural amino acid.

21. The method of claim 20 wherein said administration inhibits binding of bFGF.

22. A method of inhibiting selectins in a patient in need thereof comprising the administration of a pharmaceutically effective amount of a compound of formula I:

$$W\text{-}(X)_n\text{-}Y\text{-}[(X)_n\text{-}W\text{-}(X)_n\text{-}Y]_m\text{-}(X)_n\text{-}W \qquad (I)$$

wherein

W is independently selected from the group consisting of

a) saccharides;

b) aryl, aralkyl;

c) alkyl of 1 to 8 carbon atoms, optionally substituted with 1-2 substituents selected from the group consisting of lower aryl, lower alkyl, =O, -OR, $-NR'_2$, -SR, $-SO_4R$, $-SO_3R$ -COOR, alk-COOR; and

d) cyclic alkyl or 5-7 carbon atoms, heterocyclic alkyl of 5-7 ring atoms and 1-2 heteroatoms selected from the group consisting of N, O, and S, all optionally substituted with 1-5 substituents selected from the group consisting of =O, -OH, -OR, $-NR'_2$, -SR, $-SO_4R$, $-SO_3R$, -COOR, and alk-COOR;

Y is independently selected from the group consisting of $-NR^3\text{-}C(O)\text{-}$ and $-C(O)\text{-}NR^3\text{-}$;

X is a difunctional or polyfunctional group selected from the group consisting of

a) aryl, aralkyl;

b) alkyl of 1-8 carbon atoms, optionally substituted with 1-3 substituents in the alkyl backbone or exo to the backbone selected from the group consisting of lower aryl, lower alkyl -O-, -NR'-, -S-, =O, -OH, -OR, -NR'$_2$, -SH, -SR, -SO_4R, -SO_3R, -COOR, and alk-COOR;

each n is independently 0 or 1;

each m is independently 0 or an integer from 1 to 99 with the proviso that the total number of W groups is 2-100;

R is -H, or lower alkyl, lower aryl, and lower aralkyl;

R'is independently selected from the group consisting -H, lower alkyl of 1-4 carbon atoms, aralkyl of 2 to 19 carbon atoms, and -C(O)R";

R" is lower alkyl of 1 to 4 carbon atoms; and

R^3 is selected from the group consisting of -H, alkyl of 1-8 carbon atoms, and aralkyl of 5-8 carbon atoms; and pharmaceutically acceptable salts thereof; with the following provisos:

a) when the total number of W groups is 2, then both W groups may not be 2-amino hexoses;

b) at least one W group is a saccharide; and

c) if the terminal W is a N-acetylglucosamine, it may not be linked through -NHCO- at the anomeric carbon to a natural amino acid.

23. The method of claim 22 wherein said administration inhibits cell adhesion.

24. A method of inhibiting heparanase in a patient in need thereof comprising administration of a pharmaceutically effective amount of a compound of formula I:

$$W-(X)_n-Y-[(X)_n-W-(X)_n-Y]_m-(X)_n-W \qquad (I)$$

wherein

W is independently selected from the group consisting of

a) saccharides;

b) aryl, aralkyl;

c) alkyl of 1 to 8 carbon atoms, optionally substituted with 1-2 substituents selected from the group consisting of lower aryl, lower alkyl, =O, -OR, $-NR'_2$, -SR, $-SO_4R$, $-SO_3R$ -COOR, alk-COOR; and

d) cyclic alkyl or 5-7 carbon atoms, heterocyclic alkyl of 5-7 ring atoms and 1-2 heteroatoms selected from the group consisting of N, O, and S, all optionally substituted with 1-5 substituents selected from the group consisting of =O, -OH, -OR, $-NR'_2$, -SR, $-SO_4R$, $-SO_3R$, -COOR, and alk-COOR;

Y is independently selected from the group consisting of $-NR^3-C(O)-$ and $-C(O)-NR^3-$;

X is a difunctional or polyfunctional group selected from the group consisting of

a) aryl, aralkyl;

b) alkyl of 1-8 carbon atoms, optionally substituted with 1-3 substituents in the alkyl backbone or exo to the backbone selected from the group

consisting of lower aryl, lower alkyl -O-, -NR'-, -S-, =O, -OH, -OR, $-NR'_2$, -SH, $-SR_1$, $-SO_4R$, $-SO_3R$, -COOR, and alk-COOR;

each n is independently 0 or 1;

each m is independently 0 or an integer from 1 to 99 with the proviso that the total number of W groups is 2-100;

R is -H, or lower alkyl, lower aryl, and lower aralkyl;

R'is independently selected from the group consisting -H, lower alkyl of 1-4 carbon atoms, aralkyl of 2 to 19 carbon atoms, and -C(O)R";

R" is lower alkyl of 1 to 4 carbon atoms; and

R^3 is selected from the group consisting of -H, alkyl of 1-8 carbon atoms, and aralkyl of 5-8 carbon atoms; and pharmaceutically acceptable salts thereof; with the following provisos:

a) when the total number of W groups is 2, then both W groups may not be 2-amino hexoses;

b) at least one W group is a saccharide; and

c) if the terminal W is a N-acetylglucosamine, it may not be linked through -NHCO- at the anomeric carbon to a natural amino acid.

26. A composition comprising one or more compounds of formula I

$$W-(X)_n-Y-[(X)_n-W-(X)_n-Y]_m-(X)_n-W \qquad (I)$$

wherein

W is independently selected from the group consisting of

a) saccharides;

b) aryl, aralkyl;

c) alkyl of 1 to 8 carbon atoms, optionally substituted with 1-2 substituents selected from the group consisting of lower aryl, lower alkyl, =O, -OR, $-NR'_2$, -SR, $-SO_4R$, $-SO_3R$, -COOR, -alk-COOR; and

d) cyclic alkyl or 5-7 carbon atoms, heterocyclic alkyl of 5-7 ring atoms and 1-2 heteroatoms selected from the group consisting of N, O, and S, all optionally substituted with 1-5 substituents selected from the group consisting of =O, -OH, -OR, $-NR'_2$, -SR, $-SO_4R$, $-SO_3R$, -COOR, and -alk-COOR;

Y is independently selected from the group consisting of $-NR^3-C(O)-$ and $-C(O)-NR^3-$;

X is a difunctional or polyfunctional group selected from the group consisting of

a) aryl, aralkyl;

b) alkyl of 1-8 carbon atoms, optionally substituted with 1-3 substituents in the alkyl backbone or exo to the backbone selected from the group consisting of lower aryl, lower alkyl, -O-, -NR'-, -S-,

=O, -OH, -OR, $-NR'_2$, -SH, -SR, $-SO_4R$, $-SO_3R$, -COOR, and -alk-COOR;

each n is independently 0 or 1;

each m is independently 0 or an integer from 1 to 99 with the proviso that the total number of W groups is 2-100;

R is -H, or lower alkyl, lower aryl, and lower aralkyl;

R'is independently selected from the group consisting -H, lower alkyl of 1-4 carbon atoms, aralkyl of 2 to 19 carbon atoms, and -C(O)R";

R" is lower alkyl of 1 to 4 carbon atoms; and

R^3 is selected from the group consisting of -H, alkyl of 1-8 carbon atoms, and aralkyl of 5-8 carbon atoms;

and pharmaceutically acceptable salts thereof; with the following provisos:

a) when the total number of W groups is 2, then both W groups may not be 2-amino hexoses;

b) at least one W group is a saccharide; and

c) if the terminal W is a N-acetylglucosamine, it may not be linked through -NHCO- at the anomeric carbon to a natural amino acid.

27. A saccharopeptide combinatorial library comprising compounds of formula I

$$W-(X)_n-Y-[(X)_n-W-(X)_n-Y]_m-(X)_n-W \qquad (I)$$

wherein

W is independently selected from the group consisting of

a) saccharides;

b) aryl, aralkyl;

c) alkyl of 1 to 8 carbon atoms, optionally substituted with 1-2 substituents selected from the group consisting of lower aryl, lower alkyl, =O, -OR, $-NR'_2$, -SR, $-SO_4R$, $-SO_3R$, -COOR, -alk-COOR; and

d) cyclic alkyl or 5-7 carbon atoms, heterocyclic alkyl of 5-7 ring atoms and 1-2 heteroatoms selected from the group consisting of N, O, and S, all optionally substituted with 1-5 substituents selected from the group consisting of =O, -OH, -OR, $-NR'_2$, -SR, $-SO_4R$, $-SO_3R$, -COOR, and -alk-COOR;

Y is independently selected from the group consisting of $-NR^3-C(O)-$ and $-C(O)-NR^3-$;

X is a difunctional or polyfunctional group selected from the group consisting of

a) aryl, aralkyl;

b) alkyl of 1-8 carbon atoms, optionally substituted with 1-3 substituents in the alkyl backbone or exo to the backbone selected from the group consisting of lower aryl, lower alkyl, -O-, -NR'-, -S-,

=O, -OH, -OR, $-NR'_2$, -SH, -SR, $-SO_4R$, $-SO_3R$, -COOR, and -alk-COOR;

each n is independently 0 or 1;

each m is independently 0 or an integer from 1 to 99 with the proviso that the total number of W groups is 2-100;

R is -H, or lower alkyl, lower aryl, and lower aralkyl;

R' is independently selected from the group consisting -H, lower alkyl of 1-4 carbon atoms, aralkyl of 2 to 19 carbon atoms, and -C(O)R";

R" is lower alkyl of 1 to 4 carbon atoms; and

R^3 is selected from the group consisting of -H, alkyl of 1-8 carbon atoms, and aralkyl of 5-8 carbon atoms;

and pharmaceutically acceptable salts thereof; with the following provisos:

a) when the total number of W groups is 2, then both W groups may not be 2-amino hexoses;

b) at least one W group is a saccharide; and

c) if the terminal W is a N-acetylglucosamine, it may not be linked through -NHCO- at the anomeric carbon to a natural amino acid.

28. An array of compounds comprised of compounds of formula I

$$W-(X)_n-Y-[(X)_n-W-(X)_n-Y]_m-(X)_n-W \qquad (I)$$

wherein

W is independently selected from the group consisting of

a) saccharides;

b) aryl, aralkyl;

c) alkyl of 1 to 8 carbon atoms, optionally substituted with 1-2 substituents selected from the group consisting of lower aryl, lower alkyl, =O, -OR, $-NR'_2$, -SR, $-SO_4R$, $-SO_3R$, -COOR, -alk-COOR; and

d) cyclic alkyl or 5-7 carbon atoms, heterocyclic alkyl of 5-7 ring atoms and 1-2 heteroatoms selected from the group consisting of N, O, and S, all optionally substituted with 1-5 substituents selected from the group consisting of =O, -OH, -OR, $-NR'_2$, -SR, $-SO_4R$, $-SO_3R$, -COOR, and -alk-COOR;

Y is independently selected from the group consisting of $-NR^3-C(O)-$ and $-C(O)-NR^3-$;

X is a difunctional or polyfunctional group selected from the group consisting of

a) aryl, aralkyl;

b) alkyl of 1-8 carbon atoms, optionally substituted with 1-3 substituents in the alkyl backbone or exo to the backbone selected from the group consisting of lower aryl, lower alkyl, -O-, -NR'-, -S-,

=O, -OH, -OR, $-NR'_2$, -SH, -SR, $-SO_4R$, $-SO_3R$, -COOR, and -alk-COOR;

each n is independently 0 or 1;

each m is independently 0 or an integer from 1 to 99 with the proviso that the total number of W groups is 2-100;

R is -H, or lower alkyl, lower aryl, and lower aralkyl;

R'is independently selected from the group consisting -H, lower alkyl of 1-4 carbon atoms, aralkyl of 2 to 19 carbon atoms, and -C(O)R";

R" is lower alkyl of 1 to 4 carbon atoms; and

R^3 is selected from the group consisting of -H, alkyl of 1-8 carbon atoms, and aralkyl of 5-8 carbon atoms;

and pharmaceutically acceptable salts thereof; with the following provisos:

a) when the total number of W groups is 2, then both W groups may not be 2-amino hexoses;

b) at least one W group is a saccharide; and

c) if the terminal W is a N-acetylglucosamine, it may not be linked through -NHCO- at the anomeric carbon to a natural amino acid; wherein said compounds are attached to a synthesis support.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07H15/00 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07H A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TETRAHEDR. LETT., vol. 36, no. 11, 1995, pages 1775-8, XP002012513 K.C. NICOLAOU ET AL.: "Carbonucleotides and Carbopeptoids: New Carbohydrate Oligomers" see figure 1 ---	1-28
X	BULL. CHEM. SOC. JPN., vol. 49, 1976, pages 2511-4, XP002012514 S. TSUCHIDA ET AL.: "Aminosugars XXVI. Synthesis of Amido-bonded Disaccharides Containing Hexosaminuronic Acids" see the whole document --- -/--	1-28

[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 September 1996	27.09.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Bardili, W

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INTERNATIONAL SEARCH REPORT

Inter. nal Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEM. BER., vol. 88, 1955, pages 188-195, XP002012515 K. HEYNS AND H. PAULSEN: "Synthese der D-Glucosaminuronsäure" compound III see page 190 ---	1-28
X	CHEM. BER., vol. 108, 1975, pages 2254-60, XP002012516 E.-F. FUCHS AND J. LEHMANN: "Synthese von 7-Amino-2,6-anhydro-7-desoxy-D-glycero-L-manno-heptonsäure und 7-Amino-2,6-anhydro-7-desoxy-D-glycero-D-gulo-heptonsäure" see abstract ---	1-28
X	ANGEW. CHEM. INT. ED. ENGL., vol. 33, 1994, pages 687-9, XP002012517 V. ROEDERN AND H. KESSLER: "A Sugar Amino Acid as a Novel Peptidomimetic" see compound (9) ---	1-28
X	ANGEW. CHEM. INT. ED. ENGL., vol. 34, no. 1, 1995, pages 60-3, XP002012518 D. VETTER ET AL.: "A Versatile Solid-Phase Synthesis of N-Linked Glycopeptides" see compound (5) ---	1-28
P,X	J. CHEM. SOC., CHEM. COMMUN., vol. 23, 1995, pages 2425-6, XP002012519 H.P. WESSEL ET AL.: "Novel Oligosaccharide Mimetics by Solid-Phase Synthesis" see the whole document ---	1-28
P,X	TETRAHEDR. LETT., vol. 37, 1996, pages 1575-8, XP002012520 Y. ICHIKAWA ET AL.: "Synthesis of a New Carbohydrate Mimetics" see the whole document ---	1-28
X	METHODS ENZYMOL., vol. 247, 1994, pages 153-76, XP002012521 R. BROSSMER AND H.J. GROSS: "Sialic Acid Analogs and Application for Preparation of Neoglycoconjugates" see compound (12) --- -/--	1-28

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 96/06731

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CARBOHYDR. RES., vol. 64, 1978, pages 302-8, XP002012522 R.T. LEE AND Y.C. LEE: "Some Synthetic Inhibitors of beta-D-Glucosiduronase" see compound (7) ---	1-28
X	CHEMICAL ABSTRACTS, vol. 66, no. 15, 1967 Columbus, Ohio, US; abstract no. 65793, page 6214; XP002012526 see abstract & IZV. AKAD. NAUK SSSR, SER. KHIM., vol. 8, 1966, pages 1400-4, N.K. KOCHETKOV ET AL.: "Glycopeptides. Some Properties of Aminoacyl Derivatives of Carbohydrates with an Amide Bond" see the whole document ---	1-28
X	CARBOHYDR. RES., vol. 263, 1994, pages 181-96, XP002012523 M. SUGIMOTO ET AL.: "Total Synthesis of a Modified Ganglioside, De-N-Acetyl GM2" see 'scheme 4' ---	1-28
X	J. MED. CHEM., vol. 18, 1975, pages 771-6, XP002012524 C. COUTSOGEORGOPOULOS ET AL.: "Inhibitors of Protein Synthesis" see 'Chart II' ---	1-28
X	WO,A,93 23029 (KANTO ISHI PHARMA CO LTD ;TAKAHAMA KAZUO (JP); KAI HIROHUMI (JP);) 25 November 1993 see abstract ---	1-28
X	WO,A,95 04751 (GLYCOMED INC) 16 February 1995 see the whole document ---	1-28
X	WO,A,95 05182 (GLYCOMED INC) 23 February 1995 see the whole document ---	1-28
X	WO,A,94 03469 (FIDIA SPA ;ROMEO AURELIO (IT); KIRSCHNER GUNTER (IT); MANEV HARI () 17 February 1994 see page S ---	1-28
	-/--	

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 96/06731

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,95 10296 (GLYCOMED INC) 20 April 1995 see the whole document	1-28
X	US,A,5 369 017 (WONG CHI-HUEY ET AL) 29 November 1994 see the whole document	1-28
P,X	GB,A,2 289 274 (ERBA CARLO SPA ;PHARMACIA SPA (IT)) 15 November 1995 see the whole document	1-28
A	EXPERT OPINION ON INVESTIGATIONAL DRUGS, vol. 3, 1994, pages 709-24, XP002012525 F. DASGUPTA AND N.N. RAO: "Anti-Adhesive Therapeutics: A new Class of Anti-Inflammatory Agents" see the whole document	1-28

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/ US 96/ 06731

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Please see Further Information sheet enclosed.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Incomplete Search
Claims searched incompletely: 1-28

Annex:

The claims 1 through 28 are so broad in scope that a complete search was not possible. The Search had therefore to be limited.

A first analysis of claim 1 reveals that it comprises classes of compounds well known to the chemist. For instance, if W on the left side of formula (I) is given the meaning saccharide; the integers n and m are set to zero; W is alkyl or aryl; Y is $-NR^3-CO$, then relatively simple alkyl or aryl amido saccharides result. Examples of that type of compounds are depicted in Methods Enzymol. 247, page 159 or Carbohydr. Res. 263, page 185. Other specific examples of that type of compounds have been investigated as selectin ligands extensively in numerous publications. Other compounds that belong to the same category of compounds are mucins.

Secondly, another group of compounds covered by claim 1 belong to the class of glycopeptides. Although the applicants have attempted to limit the claims over that class of compounds, their restrictions of the claims proved insufficient. For instance, US-A-5 369 017 shows that a peptide residue may be bound to a saccharide residue that is not a simple glucosamine.

The analysis of the dependent claims related to compounds reveal that they are either too broad to allow a complete search or too narrow to allow a meaningful search. For instance, a search based on claims 3 or 4 would not be justified as they recite only very specific saccharide radicals.

Similar observations apply to the rest of claims, in

particular to the claims related to methods, compositions, libraries and arrays.

Therefore, the search had to be based on the specific disclosure given in the description of the invention. It was endeavoured to cover as much of the examples as possible, in particular those with experimental evidence on a possible therapeutic use. Hence, it was searched for compounds with a 1- (or 2-, 3-, 4-) amino uronic acid structure that contain a natural or unnatural amino acid residue linked with the uronic acid directly through its amino group. No structural restrictions have been made with respect to the amino acid residue. It may be natural, artificial or part of a peptide. Neither have restrictions been made on the stereochemistry of the uronic acid; it may be a glacturonic acid, glucuronic acid etc.; it may also bear substituents. So it was possible to cover all compounds of the type illustrated on page 91; 97; 103; 108; 111; 116 (left col.).

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/US 96/06731

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9323029	25-11-93	JP-A-	5320046	03-12-93
		AU-B-	4088393	13-12-93
WO-A-9504751	16-02-95	US-A-	5508387	16-04-96
		AU-B-	7553094	28-02-95
		CA-A-	2168454	16-02-95
		EP-A-	0720619	10-07-96
WO-A-9505182	23-02-95	AU-B-	7520394	14-03-95
WO-A-9403469	17-02-94	IT-B-	1260156	28-03-96
		AU-B-	4801493	03-03-94
		CA-A-	2141679	17-02-94
		EP-A-	0652887	17-05-95
		JP-T-	8502953	02-04-96
WO-A-9510296	20-04-95	CA-A-	2173990	20-04-95
US-A-5369017	29-11-94	AU-B-	1254695	21-08-95
		WO-A-	9521262	10-08-95
GB-A-2289274	15-11-95	NONE		